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THE CONTRACTION RATE OF THE EXCISED RAT UTERUS WITH REFERENCE TO THE OESTROUS CYCLE

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The investigation which this report covers has been made possible by the accurate work of Long and Evans (5) on the oestrous cycle of the rat. It is an attempt to find some correlation between the physiological condition of the uterine muscle and that of other parts of the genital tract throughout the oestrous cycle.

The occurrence of rhythmic contractions of the excised uterus when properly treated was described by Kehrer (3). His method has been adopted for the present study. He also described a difference in the form and amplitude of these contractions corresponding with muscular hypertrophy of pregnancy. Langley (4) has shown that the effect of certain drugs, e.g., adrenalin, on the excised pregnant uterus differs in some species from the effect on the non-gravid organ. Some observations on the pseudo-pregnant uterus (5) tend to show a parallelism, with the behavior of the pregnant organ which may be included in a later paper. But, so far as we have been able to ascertain, no work has been reported which attempts to trace any change in the action of the uterine muscle referable to the oestrous cycle.

Two general groups of experiments were performed. In the first, the preparation used was a strip of uterus cut free from the mesometrium and from the cervix and ovary. It was ligated at both ends and suspended in a bath of warm (38°C.) oxygenated Locke's solution in either the apparatus of Dale and Laidlaw (2) or in the simple apparatus described by Kehrer. Contractions were recorded through a heart lever on a long roll kymograph moving at a rate of about 3 cm. per

minute. In some cases both horns of the uterus were set up in parallel and contractions recorded at the same time. In these cases the mean of the contractions rates of the two horns was taken as the rate for the individual.[1] Eighteen experiments of this type were reported in our preliminary note (1).

In the second group of experiments the entire genital tract from vulva to mesovarium was dissected out with the minimum exposure to cold and trauma. The preparation was set up in the Kehrer bath, with Englemann heart pinzette attached to the bottom holding the vagina, and two pinzettes fastening the mesovarial fat to two heart levers. All the animals were killed by crushing the neck in a large Kelly hemostatic forceps, and were carefully dissected as quckly as possible.

The experiments of both groups fall also into three age groups. The first is composed of sexually immature animals in which ovulation had not occurred, some of which were used before the opening of the vaginal orifice. The second group was composed of young, or adolescent animals in which the oestrous cycle was being followed by Long and Evans' method (5) of examining the vaginal cell content. The upper limit of this group was set roughly at the age of 3 months and the weight at 200 grams. No animals which had borne litters were included in this group. The third group included both virgin and parous animals over 3 months in age or about 200 grams in weight. A history of previous pregnancy had no apparent effect on the contraction rate.

Our analysis of the records leaves much to be desired. Contractions were observed which apparently involved all or nearly all the muscular tissue of the uterus in a maximal or nearly maximal effort. Others were observed which were barely perceptible. Nearly all gradations of amplitude were observed between the maximal and barely perceptible contractions. The maximal contractions alone were first counted and reduced to rate per hour. Then the total number of all contractions *irrespective of amplitude* was reduced to an hourly rate. Both rates are compared in the accompanying tables.

What constitutes a maximal contraction is not easy to say in some records. Counts made by different workers or by the same man at different times disagree within narrow limits. The fact that all the counts were made by one person tends to eliminate error due to personal equation, as this was a possible source of error in tracings of all

[1] The rates for the two horns were rarely the same. They often differed from each other by 25 per cent or even more.

classes of the experiments. It is possible that two or more rhythms interfering according to physical laws of harmony are present in the organ. Some tracings suggest this rather strongly. It can scarcely be imagined in others.

TABLE 1

Mean contraction rates

EXPERIMENT	RATE PER HOUR					
	Maximal contractions		Submaximal contractions		All contractions	
	Dioestrous interval	Oestrous	Dioestrous interval	Oestrous	Dioestrous interval	Oestrous
Immature animals, minimum trauma	28.0		28.0		56.0	
Number of experiments	3.0		3.0		3.0	
Immature animals, strip preparations					78.0	
Number of experiments					3.0	
Young adults, minimum trauma	25.5	17.3	26.5	35.2	52.0	52.5
Number of experiments	5.0	10.0	5.0	10.0	5.0	10.0
Young adults, strip preparations	41.5	32.0	34.0	33.0	75.5	65.0
Number of experiments	3.0	7.0	3.0	7.0	3.0	7.0
Old adults, minimum trauma	31.0	18.0	42.3	36.6	73.3	54.6
Number of experiments	6.0	5.0	6.0	5.0	6.0	5.0
Old adults, strip preparations	55.4	31.0	27.0	31.6	82.4	62.6
Number of experiments	10.0	14.0	10.0	14.0	10.0	14.0
All adults, minimum trauma	28.5	17.5	35.1	35.7	63.6	53.2
Number of experiments	11.0	15.0	11.0	15.0	11.0	15.0
All adults, strip preparations	52.2	31.3	28.6	32.1	80.8	63.4
Number of experiments	13.0	21.0	13.0	21.0	13.0	21.0
All adults, both methods	41.4	25.6	31.6	33.6	73.0	59.2
Number of experiments	24.0	36.0	24.0	36.0	24.0	36.0

Another interesting point which was strongly suggested by some experiments was the origin of the contraction wave. In some of the experiments it was noted on the tracing at the time. If the origin and direction of the contractile impulse could be shown to differ in the different stages of the oestrous cycle, the fact might be very significant.

It was not noted in enough of our experiments to justify any conclusions. Furthermore, the observations were not consistent, and it is often impossible to judge reliably the source of a contraction wave by naked eye inspection. Electrical methods which have told us so much about cardiac physiology and which are beginning to throw light upon gastro-intestinal physiology, seem to be indicated for the approach to this phase of our problem.

Finally, the question as to what relation the behavior of the uterus subjected to the trauma and tension incident to the method bears to its behavior *in vivo* is still open. Wysenbuk's observations on the rabbit uterus (6) through transparent windows placed in the abdominal wall failed to show any movements of the non-pregnant uterus comparable to those seen in the excised organ. However, this question must remain unanswered for the present or until our methods of investigation are improved.

The findings in these experiments are tabulated herewith. In table 1 the mean contraction rates are collected in one summary. Acting on a suggestion of Dale and Laidlaw (2) that the excised uterus of the young virgin guinea pig is more sluggish than that of older animals, we performed six different experiments on immature animals, the smallest of which weighed 86 grams and the youngest of which was 35 days old. In every case we found a fairly rapid rate of contraction. We also found tonus oscillations, sometimes of greater amplitude than the strongest single contractions. This phenomenon was seldom found in ovulating animals and never to such a marked degree as in the immature animals.

The most sluggish rat uteri which were observed were those of young animals at oestrus during the first few cycles. This fact suggested the division of the sexually mature animals into a younger and an older group. The limit set (3 months) was arbitrarily chosen in late adolescence. The contraction rates in the older group are, on the whole, higher than in the younger group, but this difference is not considerable. Consequently, the mean rates are tabulated irrespective of age as well as in age groups. The contractions fall naturally into two groups. The first is composed of presumably maximal contractions which are of approximately constant amplitude for any given strip. The second group is the submaximal contractions, including many which are barely perceptible.

The most outstanding single fact observed is that *the rhythm of the maximal contractions is much slower at oestrus than during the dioestrous*

interval, or resting stage. The uterus at oestrus is distended with fluid, but experiments which I have made on the resting stage uterus distended with Locke's solution, and on the uterus at oestrus with the fluid let out indicate that the mechanical fact of distention is not a factor in causing the difference of rhythm. The submaximal contractions do not show this pronounced difference of rate. The mean rate, averaging all experiments, is *slightly* higher during oestrus. If the mean rate of all contractions, including the minimal and maximal, be taken, the relation is similar to that found for the maximal contractions. The main observation (that the contraction rate of the uterus excised during oestrus is slower than during dioestrus) is equally true for both age groups and for both methods of making the preparations. In all four groups of experiments, however, there was overlapping of the rates. The highest rate found at oestrus was higher than the lowest rate found during the dioestrous interval. Consequently, our conclusions are drawn from averages. The mean contraction rate for maximal contractions in all animals killed during oestrus is only 62 per cent of the mean rate in the animals killed during dioestrus.

SUMMARY

The uterus of the rat was studied with reference to the oestrous cycle, by the well known method of Kehrer. The *mean* contraction rate, considering maximal contractions, was found to be definitely lower during oestrus than during the dioestrous interval. The contraction rate for submaximal contractions varied between wide limits but the mean rate was little affected by the events of the oestrous cycle.[2]

[2] While this paper was in press Keye at Johns Hopkins University (Bull. Johns Hopkins Hosp., xxxiv, 60, 1923) published the report of a similar line of study on the uterus of the domestic sow. His preparations were so made as to record the contractions of the circular muscle layer. Ours give the contractions of the longitudinal layer, and by naked eye observation the course of the peristaltic wave in the circularis could sometimes be noted. Our observations of this sort were inconclusive, both peristalsis and antiperistalsis being observed in the same preparation and the direction often being indiscernible. Keye found that the major rhythm (corresponding apparently to our maximal contractions) was at its height during oestrus and was gradually replaced by a minor rhythm of greater frequency but less amplitude during the time in dioestrus when the ova are in the uterus. In our experiments we find the maximal contractions most rapid during the time (dioestrus) when the ova are in the uterus, and the submaximal contractions slightly less frequent at this time.

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(5) LONG AND EVANS: Memoirs of the University of California, 6, Univ. of Cal. Press, 1922.
(6) WYSENBUK: Nederlandsch. Tydschr. v. Geneeskunde, 1922, I, 1263 (cited in abstract only).

Keye suggests that the presence of several sets of corpora lutea in the rat's ovaries at the same time may account for the discrepancies between the behavior of the rat's and pig's uterus. However, in young rats during their first cycle when only one set of corpora is present we have found the same phenomena as in the older animals with many sets of corpora present.

Until more is known of the relation of the behavior of the excised uterus to its behavior in vivo we prefer not to comment on his explanation of the possible purpose and function of these uterine contractions.

THE EFFECT OF DEFICIENT AND EXCESSIVE PULMONARY VENTILATION ON NASAL VOLUME

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In a study of nasal plethysmographic changes subsequent to various procedures, we had occasion to study the effects of partial asphyxia and of over-ventilation.

Dogs and rabbits were used as experimental animals, the former under barbital, the latter under urethane anesthesia. The nasal plethysmograph arrangement of Tschalussow[1] was at first used.

If, in such a preparation in which a good pulse record is obtained, indicating a sensitive arrangement, one obstructs the tracheal cannula, there results a prompt enlargement of the nasal chambers, with occasionally some accompanying change in blood pressure. On the other hand, if one produces over-ventilation by positive pressure methods so as to lead to apnea the nasal plethysmogram indicates vasodilatation. Blood pressure changes are in this instance frequently complicated by the increased intrapulmonic pressure interfering with filling of the heart.

The same vasoconstriction produced by obstruction-asphyxia is produced by causing the animal to breathe carbon dioxide in sufficient amounts as to greatly increase respiratory activity.

In view of a number of possible explanations of the changes above shown, the most obvious seemed to be *a*, that the vasoconstriction is nervous, i.e., a type of reflex involving the cervical sympathetic nerves, *b*, systemic changes in circulation in which case the nasal vessels would respond passively to other circulatory changes.

In order to determine the origin of the vasoconstriction observed in partial asphyxia, the cervical sympathetic nerves were divided in an animal after having obtained satisfactory control effects by asphyxia. In some animals section of the vago-sympathetic nerves was done. In

[1] Tschalussow, M. A., Arch. f. d. gesammt. Physiol., 1913, cli, 523.

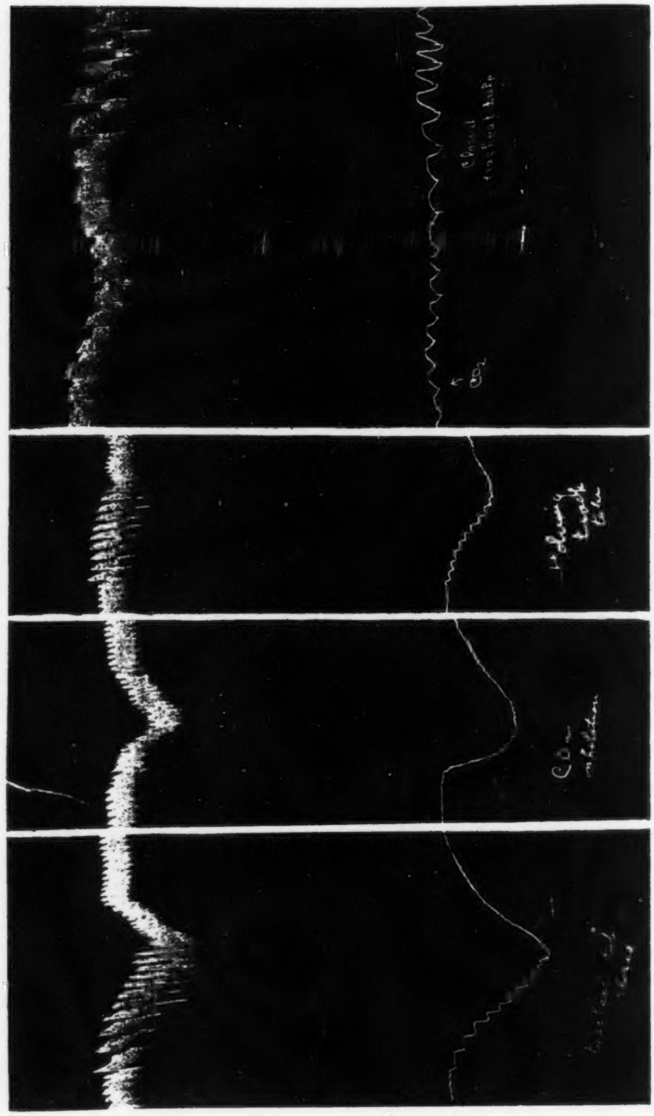

Fig. 1 Fig. 2 Fig. 3 Fig. 4

Figs. 1–4. Dog, barbital anesthesia. Upper tracing, blood pressure; lower tracings, plethysmogram.
1 and 3. Effect of obstruction of tracheal tube.
2. Effect of CO_2.
4. Effect of obstruction and CO_2 after section of the cervical sympathetic nerves.

both instances the nerve section abolished the prompt vasoconstriction response of the nasal mucous membranes to asphyxiating conditions. The vasodilatation on over-ventilation appeared usually to be essentially the same as before the division of the cervical sympathetic nerves. Likewise the blood pressure change was essentially the same as before nerve section.

We next performed subjective experiments on ourselves. One may subjectively measure or graduate the resistance offered by the nasal membranes to the passage of air during normal quiet breathing. The subjective measurement is made again after having held the breath till near the "breaking point." There is thereby developed a sense of diminished resistance which we interpret to mean vasoconstriction in the nasal membranes. Partial asphyxia by rebreathing from a rubber bag produces the same effect. Conversely by over-ventilation there was felt a very distinct sense of increased resistance to air passage. We have submitted these tests to many individuals, none of whom knew whether to expect an increase or decrease of resistance and invariably they have reported subjective sensations completely confirming our own observations. An additional subjective experiment is pertinent. By holding one's breath in various phases of respiration, e.g., at beginning or end of expiration or by making a forced expiratory or inspiratory effort against the closed glottis, we obtained but one result, namely, a distinct sense of diminished resistance to air passage.

These subjective experiments are in complete conformity with the objective experiments described above. Objective experiments on ourselves we have performed by the methods described below which are more convenient and we believe less subject to reflex complications than the Tschalussow technique in man, though Tschalussow used his technique on himself and was quite successful.

The methods used in these objective experiments in human nasal plethysmography we believe are new and based on somewhat different principles from those used ordinarily in plethysmography.

a. By connecting a tube, held tightly in one nostril, to a recording tambour one can record changes in resistance to air passage offered by the patent nasal passage.

b. By passage of a continuous stream of air under constant pressure into one nasal chamber via one nostril the inflowing air will find its way out through the other nasal chamber or into the nasopharynx and out through the mouth. If the mouth and glottis both be closed a constant stream of air passes out through the unobstructed nasal chamber. A

change in caliber of the nasal chambers will change the resistance to air flow and hence can be recorded by means of a tambour connected to the inflowing air connection by means of a T tube. In man, momentary insertion of the nasal tube, preferably of fairly large pressure tubing, permits the recording of relative resistance with considerable consistency. On repetition of the test subsequent to asphyxia, physical exercise or over-ventilation, changes in nasal resistance are readily demonstrated. In laboratory animal experiments by this latter method, it is well to insert a tube into the unobstructed nostril and also a plug of cotton in the nasopharynx to prevent changes due to naris or soft palate movement.

We have used both these methods on ourselves and the second (positive pressure method) in both rabbits and dogs breathing through a tracheal cannula. By these two new methods we have positive objective confirmation that the subjective sensations described above are based on actual changes in air resistance through the nasal passages. During the time of voluntary apnea a dilatation of the nasal vessels may occur lasting till near the "breaking point" when constriction occurs. A measurement of nasal resistance after two or three full breaths shows a distinct lessening of resistance when compared to the control tests.

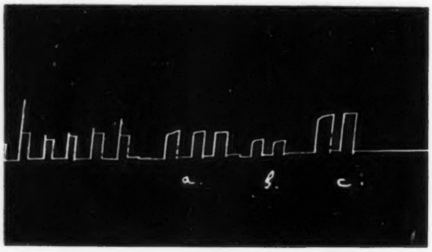

Fig. 5. Human nasal plethysmogram using positive pressure method. *a*, control; *b*, effect of partial asphyxia; *c*, effect of over-ventilation.

Passive changes in the mucous membranes subsequent to circulatory changes such as changes in heart rate or force and changes in intrathoracic pressure on blood flow through the lungs and large veins to the heart do not account for the observations recorded. Passive changes should still be present after sympathetic nerve section. Over-ventilation in ourselves—leading to a degree of acapnia—leads to increased resistance which we interpret to mean vasodilatation. In man, rebreathing from a rubber bag causes increased respiratory efforts paralleling the mechanical features of over-ventilation, yet in spite of this, vasoconstriction occurs in the nasal membranes. In the dog, excessive respiratory activity due to inhalation of carbon dioxide

also leads to an enlargement of the nasal chambers. In both conditions the mechanical circulatory effects from increased respiratory efforts should be more or less alike yet the nasal membrane vessels respond in a directly opposite manner.

The seat of action is doubtless the vasomotor center. Asphyxia and especially exercise may increase to some extent systemic blood pressure to which the nasal vessels may likely contribute their proportional part. Yet the nasal vessels are found to constrict regardless of whether the systemic blood pressure rises or falls on asphyxia. The significant feature, then, consists in the widening of the nasal air passage in conditions such as require increased ventilation of the blood.

SUMMARY

1. New methods of nasal plethysmography applicable alike to man and laboratory animals are described.

2. Volume changes in the nasal chambers are reflex in character. Variations in respiratory efficiency produce a change in nasal resistance to air passage acting as an adaptive reflex mechanism lessening resistance when respiratory need is increased, and possibly by a more passive mechanism increasing resistance in the converse condition.

ELECTROMYOGRAPHIC STUDIES OF MUSCULAR FATIGUE IN MAN

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The study of muscular fatigue is so complicated by psychological factors that a method of accurately recording any of its concomitant phenomena may be of value. The present study was undertaken because certain observations made in the course of other experiments indicate that the previous work in this field was not adequate.

Piper (1), (2) in 1909 and 1912 published extensive studies of the action-currents of skeletal muscle in man, and among his other observations noted that the action-currents of a fatigued muscle, as recorded by the string galvanometer, showed a slowing of rhythm. His experiments were carried out by causing the subject to grip a dynamometer as long as possible, even until the effort caused considerable pain, the action-currents being led off from the flexor muscles of the forearm. The myograms figured in his paper show that the usual frequency of action-currents during the voluntary contractions of a muscle is about 50 per second, whether the dynamometer is pressed strongly or weakly. During marked fatigue, however, this rate falls to 35 and even to 20 or 25 per second. The individual excursions of the string, which are recorded as waves on the film, are not increased in amplitude, and between the main waves (Hauptwellen) there are often short periods when no action-currents are recorded (Wellenfreie Intervalle). It is probable that his method did not elicit any increase in amplitude of the individual waves because merely pressing against a spring does not call forth any uniform amount of work and, as fatigue supervenes, less and less tension may be exerted by the muscles involved. Piper himself shows (2, p. 85) that with less muscular tension these action-currents are less and hence cause smaller excursions of the galvanometer string. Therefore it seemed that to study this problem fairly an apparatus should be devised which would call forth an approximately equal amount of

muscular contraction both at the beginning and at the end of the period of work. In other words, the dynamometer may allow the subject to relax his grip when fatigue begins to cause pain, but such an apparatus as an ergograph makes necessary a measurable amount of work at the moment of recording the action-currents.

METHOD. Muscular fatigue of the flexors of the wrist (principally the flexor carpi radialis) was studied in ten different individuals. All were young healthy adults; eight were males and two females. In the first eight experiments the isotonic contraction was used. This was accomplished by constructing a large ergograph to which the right arm was strapped, the forearm being horizontally fixed, and the lower part of the humerus being strapped to an upright board by a band around the belly of the biceps. The elbow was thus fixed at a right angle, and had practically no motion. Both the horizontal and upright boards were thickly padded with felt, so that the subjects underwent as little discomfort as possible. The hand was inserted into a heavy woolen glove, thickly coated with plaster-of-Paris, so that the finger and metacarpal joints were immovable. This so fixed the limb that motion was possible only at the wrist. A wire was attached to the plaster glove, and this ran over a pulley to the weight which hung below. In order that this motion at the wrist might be as uniform as possible a wedge-shaped block of wood was placed under the dorsum of the hand in the glove, so that when each pull began the hand was at an angle of about 30 degrees with the table-top, and ended, when the maximum lift was accomplished, with the hand at an angle of nearly 90 degrees with the horizontal. The result of this arrangement was that any motion of the wrist was opposed by the constant pull of the weight.

The action-currents were led off by placing non-polarizable electrodes (such as are described by Piper (2), Forbes (3) and Cobb (4)) on the arm so as to make a circuit through the contracting muscle and the galvanometer string. In order to accomplish this one of the electrodes was inserted in a hole in the strap over the belly of the flexor carpi radialis, in such a way that it was kept in firm contact with the skin in spite of the motion. This is spoken of as the "proximal electrode" and is connected with the upper end of the string. The "distal" or "indifferent" electrode was placed on the skin just above the elbow on the lateral aspect of the arm. It is desirable that this electrode be as far as possible from any contracting muscle, since action-currents from one muscle only are to be recorded. Since the elbow was fixed it is probable that no action-currents were developed in the triceps or biceps, so those that were

recorded arose entirely in the flexors of the wrist. The electrodes were connected with the galvanometer, completing a circuit, and the oscillations were recorded on a rapidly moving film. In experiments 1 to 8 the galvanometer used was of the Cambridge type; a complete description of the apparatus is given by Forbes and Gregg (3). The last ten experiments were made with a Hindle instrument with an air gap of 1.5 mm. The recording camera has been described by Forbes and Thacher (5).

Various strings were used, as noted in the protocols. String "G" was a fiber of gilded quartz with a diameter of 2.5 micra and a resistance of 20,000 ohms. String "B" was of platinum, diameter of 5 micra and a resistance of 910 ohms. String "H" was made of gilded quartz, its diameter being 2.5 micra and its resistance 12,000 ohms. String "2" was of gilded quartz with a diameter of 1.5 micra and a resistance of 25,000 ohms; string "no. 5" was of gilded quartz 1.75 micra in diameter with a resistance of 21,000 ohms; while "no. 6" is a similar string of 2 micra diameter and 4,800 ohms.

Besides recording the movements of the galvanometer string on the moving film a lever was so arranged that it cast a shadow on the film, simultaneously recording the mechanical movement of the wrist in lifting the weight. This lever worked against a light spring, not strong enough to add appreciably to the work imposed on the muscle. This record is referred to in the protocols as the "mechanogram" as opposed to the record of the action-currents—the "electromyogram."

The mechanogram is seen in the figures as a long slow curve; at times there are small waves superimposed upon it; these are due to vibrations set up by the rhythmic pulling on the wire and spring. The records of the mechanogram and electromyograms are synchronous and indicate simultaneous mechanical and electrical phenomena.

In the last ten experiments isometric muscular contractions were studied. The arm was fixed as before, but the hand was also strapped down firmly, and the subject was told to "pull as hard and steadily as possible" against the immovable strap. In this way fatigue was more quickly accomplished, but as no way of recording the amount of work was possible, it lay with the subject to keep pulling as hard as possible to the end of the experiment. The action-currents were led off from the flexor carpi radialis as in the isotonic experiments.

The string tension is expressed in "meters per ampere" in each protocol, this being a convenient standard of measurement which is independent of magnification and string resistance (6).

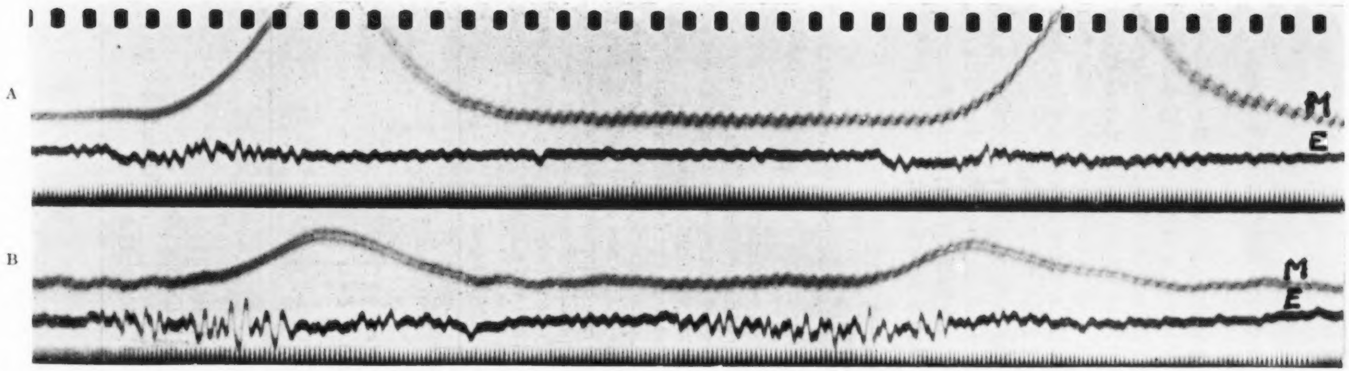


Fig. 1. Sample records from fatigue experiment with ergograph, showing simultaneous mechanogram *M*, and electromyogram *E*. Time is marked with tuning fork, vibrating at 100 D.V. per second. (Exper. 2.)

A. Record at beginning of work; high curve of mechanogram represents 22.9 centimeter-kilograms of work at each lift. Frequency of action-currents is 45 per second; average amplitude of the main waves is 3.1 mm. (Exper. 2, string B at tension of 75 meters per ampere, work and electrodes same as in exper. 8 q.v.)

B. Ninety-eight seconds later with fatigue approaching the point where weight cannot be lifted; only 10.1 centimeter-kilograms of work are done per lift; frequency of action-currents has fallen to 37 per second; average amplitude of waves has risen to 5.1 mm.

In most of the experiments the resistance of the subject is measured before and after work. To do this the circuit is closed through the tissues. A voltage from the potentiometer in the compensating circuit is selected which, when the latter circuit is closed, will deflect the string by a convenient number of millimeters, and this deflection is noted. A resistance is then found which, when substituted for the subject, will permit the same deflection for the same voltage.

Observations. Using the method above described eighteen experiments were performed on ten different individuals. The data obtained are summarized in table 1, and four typical protocols are published in full with their figures tabulated as follows:

Experiment 2. Date—October 28, 1919.
Subject—S. C. (Male, 33 years).
Work—Consists of lifting a weight of 3.9 kilos every 2 seconds at first, increasing to every second.
Weight—of 3.9 kilos.
String—B at tension of 75 meters per ampere.
Proximal electrode—over belly of flexor carpi radialis.
Distal electrode—external aspect of lower arm just above elbow.
Resistance of preparation—before work not taken.
Resistance of preparation—after work equals 10,000 ohms.
Report of subject: "Began with raising weight every 2 seconds, but difficult to follow watch after arm began to fatigue and cause pain. At this time contractions became faster and reached rate of approximately every second. Fatigue at end caused considerable pain in whole forearm, especially the muscles involved (upper forearm flexors). This was a sickening ache. There was also rather profuse general sweating of forehead and axillae."

Experiment 2

Time from beginning of work in seconds	Number of observations, i.e., contractions	Frequency of A. C. per second (main waves)	Average amplitude of A. C. in mm.	Altitude of mechanogram in mm.	Work per contraction in cm. kilos	Power = $\frac{\text{kilogram cm.}}{\text{seconds}}$	Notes
0	4	40	3.1	18	22.9	89.0	
30	5	41	3.0	14	17.8	74.0	
60	5	40	3.3	13	16.5	71.5	
90	13	37	5.1	8	10.15	41.0	
100							Exhausted, given 5 seconds rest
105	1	38	4.4	8	10.15	41.0	

Experiment 8: Date—May 6, 1920.
Subject—O. R. F. (male, 27 years).
Work—Consists of lifting, a weight of 3.9 kilos every second.
Weight—of 3.9 kilos.
String—H, at tension of 158 meters per ampere.
Proximal electrode—over forearm flexors.
Distal electrode—over external aspect of lower humerus.
Resistance of preparation—before work equals 11,000 ohms.
Resistance of preparation—after work equals 10,000 ohms.
Resistance of preparation—after rest (control) equals 10,000 ohms.
Report of subject: "Felt slight fatigue at 90 seconds, more at 120 seconds and changed position of arm slightly. At 180 seconds there was some pain, and at 300 seconds stopped with fatigue."

Experiment 8

TIME FROM BEGINNING OF WORK IN SECONDS	NUMBER OF OBSERVATIONS, I.E., CONTRACTIONS	FREQUENCY OF A. C. PER SECOND (MAIN WAVES)	AVERAGE AMPLITUDE OF MAIN WAVES IN MM.	ALTITUDE OF MECHANOGRAM IN MM.	WORK PER CONTRACTION (IN CM. KILOS)	POWER	NOTES
0	5	52	2.1	15.0	19.0	77.0	See fig. 2-A
30	1	54	1.8	11.0	14.0	62.0	
60	1	52	2.1	11.0	14.0	59.0	
90	2	47	2.5	10.0	12.7	54.0	Slight fatigue
120	3	48	4.4	8.0	10.2	47.5	More fatigue
150	4	41	4.1	8.5	10.8	52.0	
180	4	40	4.4	10.0	12.7	41.0	Some pain
235	10	42	5.1	8.0	10.2	47.5	See fig. 2-B
270	9	44	4.1	3.0	3.8	15.3	
							Rest of 6 minutes
660	6	58	1.8	13.0	16.5	68.5	See fig. 2-C
690	4	56	2.0	10.0	12.7	41.0	

Experiment 11: Date—June 18, 1921.
Subject—S. C. (male, 33 years).
Work—Consists of pulling steadily and as hard as possible against a strap. The arm is bound down and across the palm of the hand is another strap; against this the subject is told to raise the hand by flexing the wrist as strongly, and for as long a time, as possible.
String—no. 2 at tension of 306 meters per ampere.
Proximal electrode—Over belly of flexor carpi ulnaris.
Distal electrode—Over wrist tendons.
Resistance of preparation not taken.

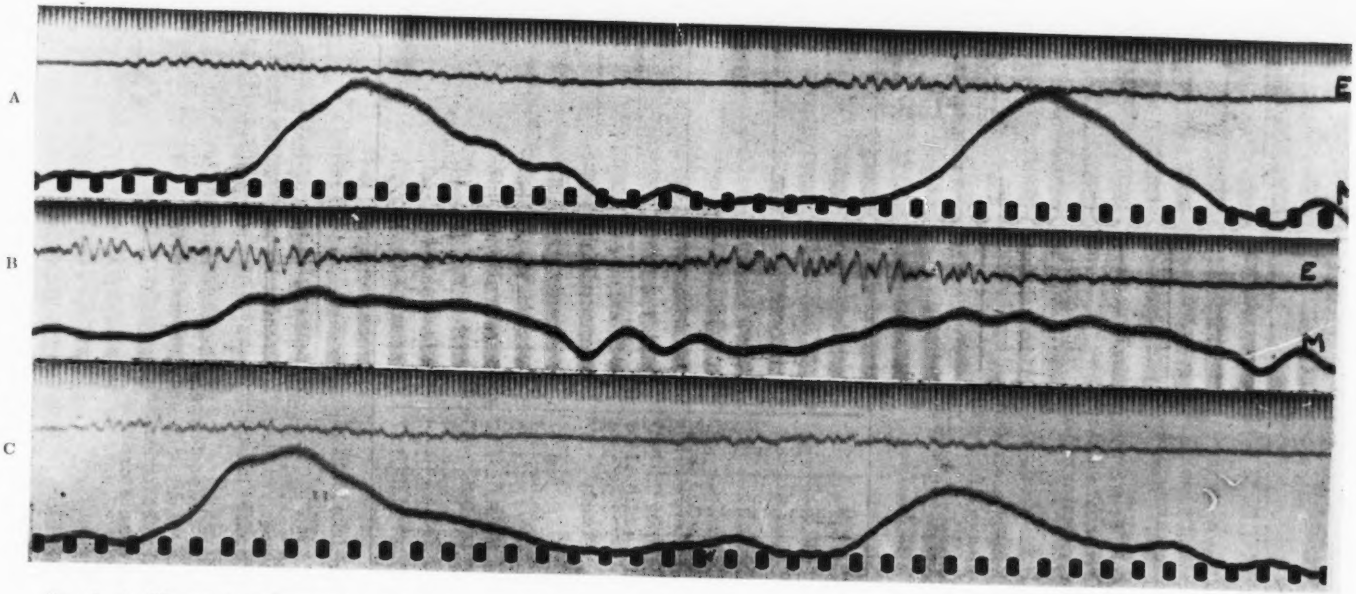


Fig. 2. *A*. (Exper. 8, see protocol.) ***Record made at beginning of work***, high curves of mechanogram indicate 19 centimeter-kilograms of work per contraction. Frequency of action-currents is 52 per second; it is of interest that these action-currents begin an appreciable time before the mechanical contractions of the muscle show by the rising curve of the mechanogram. Amplitude of the main waves of the action-currents averages 2.1 mm.

B. *After 235 seconds* of lifting the ergograph weight every second, altitude of the mechanogram is less and only 10.2 centimeter-kilograms of work are done per contraction. The frequency of action-currents has fallen to 42 per second and the amplitude of the main waves has risen to 5.1 mm. (Exper. 8, cont.)

C. *After a rest of 6 minutes* work is started again at approximately 16.5 centimeter-kilograms per contraction; the frequency of action-currents is 58 per second and the amplitude 1.8 mm. (Exper. 8, concluded.)

Report of subject: "Slight fatigue felt at 30 seconds; more at 45, and great fatigue with pain and inability to pull any more at 63 seconds after onset. The pain subsided immediately on stopping the muscular work and when the control was tried one minute later there was no discomfort."

Experiment 11

TIME FROM BEGINNING OF WORK IN SECONDS	FREQUENCY OF A. C. PER SECOND (MAIN WAVES)	AVERAGE AMPLITUDE OF A. C. IN MM.	NOTES
0	58	4.4	See fig. 3-A
15	44	6.0	
30	40	6.5	Slight fatigue. See fig. 3-B
45	36	8.0	More fatigue
60	32	9.5	Painful fatigue. See fig. 5-C
			Rest of 60 seconds
120	52	5.0	See fig. 3-D

Experiment 16: Date—November 17, 1922.
Subject—S. C. (male, 34 years).
Work—Isometric, as in experiment 11.
String—Number 5 at tension of 250 meters per ampere.
Proximal electrode—as in experiment 11.
Distal electrode—as in experiment 11.
Resistance of preparation—before work equals 110,000 ohms.
Resistance of preparation—after work equals 110,000 ohms.

Experiment 16

TIME FROM BEGINNING OF WORK IN SECONDS	FREQUENCY OF A. C. PER SECOND (MAIN WAVES)	AVERAGE AMPLITUDE OF A. C. IN MM.	NOTES
0 to 5	66	3.5	
30	50	4.0	Began to perspire
45	50	4.5	Painful fatigue begins
60	45	4.5	
70	45	4.5	Very marked fatigue
78	45	3.5*	Exposure to end, at 80 seconds
80			End of contraction
			Rest of 2 minutes 40 seconds
240	64	3.5	Control

* Failing.

The first column, headed "time from beginning of work in seconds" shows the duration of the experiment, and the time from the beginning at which each observation was made. The second column, headed "number of observations" shows how many times the weight was lifted

during the photographic exposure. In other words, five contractions were recorded at the beginning of experiment 8 starting with the first pull at time 0, but necessarily lasting for 4 seconds since the liftings of the weight were timed to be 1 second apart. Longer exposures were made at the beginning and end of the experiments because the action-

TABLE 1

EXPERIMENT	SUBJECT	TIME IN SECONDS TAKEN TO CAUSE FATIGUE	CHANGE IN FREQUENCY OF ACTION-CURRENTS	CHANGE IN AMPLITUDE OF MAIN WAVES	TOTAL AMOUNT OF WORK IN CM. KILOS	CHANGE IN RESISTANCE IN OHMS	
			per secd.	*per cent*			
							Isotonic
1	T.	150	− 7	+127	688		Isotonic
2	S. C.	90	− 4	+ 70	1005		Isotonic
3	S. C.	190	−10	+ 51	1458	10,000 to 10,000	Isotonic
4	A. F.	390	− 1.5	− 0	4080	17,000 to 18,000	Isotonic
5	O.	255	−14	+ 80	2760		Isotonic
6	B.	405	−10	+100	4387	45,000 to 30,000	Isotonic
7	S. W.	330	−18	+ 67	1431	11,000 to 10,000	Isotonic
8	O. R. F.	300	−10	+183	3300	11,000 to 10,000	Isotonic
Average........		264	− 9	+ 85			Isotonic
9A	S. C.	95	−24	+ 43			Isometric
9B	S. C.	60	−20	+ 24			Isometric
10	S. E. H	120	− 7	+150			Isometric
11	S. C.	60	−26	+116			Isometric
12	H. W. S.	75	−18	+100			Isometric
13	H. B. C.	75	−24	+ 80			Isometric
14	S. C.	70	−10	+ 75			Isometric
15	S. C.	65	−24	+111			Isometric
16	S. C.	80	−21	+ 29		110,000 to 114,00	Isometric
17	S. E. H	50	−18	+ 56		96,000 to 103,00	Isometric
18	A. F.	75	−23	+ 25		40,000 to 48,00	Isometric
Average........		75	−20	+ 73			Isometric

currents at these times were expected to show greater differences. The third column contains the "frequency of action-currents per second (main waves)," that is, the oscillations of the string recorded on a moving film appearing as "waves." When a good record is obtained

these waves run rhythmically and can be easily counted, but at times many small waves complicate the pictures. Since the significance of these small waves is not well understood only the main waves have been counted in these experiments. This counting is done by taking that part of the electromyogram which corresponds with the pull on the ergograph (the ascent of the mechanogram) and counting the number of waves per second at the point where they seem to be most regular (fig. 1).

The "average amplitude of main-waves in millimeters" as recorded in column 4, is simply the measure of the height of these waves on the film, the waves to be measured being chosen as above described under "frequency." The altitude of the mechanogram is also a simple measurement, and represents a definite proportion of the distance the weight is raised at each pull. Thus the "work" done at each contraction is figured and entered in column six, and by figuring in the angle of the mechanogram the "power" is determined.

In the isometric experiments no mechanical record could be made, so the data are simply a record of the frequency and amplitude of the action-currents at various times during the prolonged fatiguing contraction. Experiments 11 and 16 are chosen as typical.

Discussion. In looking over the various protocols presented above and comparing their data, as is done in table 1, it is seen that two changes in the electromyogram usually appear with fatigue. In the first place the frequency (rate per second) of the action-currents is decreased, as was observed by Piper (2), who also points out the lengthened periods between the waves as a factor in this slowing. This occurs in some of our records (see fig. 3C) but not in all. There is usually a distinct increase in the amplitude of the waves, indicating that action-currents of greater voltage are produced in the fatigued muscle. Piper did not observe this in his experiments, stating (p. 123) "Man findet kleine Amplitude der Hauptwellen, grosse Wellenlange und zahlreiche superponierte Nebenzacken." In his figure 40, however, the places where there is slowing of the waves also show increase in their amplitude. The fact that this phenomenon was not regularly observed by Piper may be due to the method used, for Piper caused fatigue in his subjects by having them grip strongly on a dynamometer. This pressure against a spring cannot be controlled in such a way as to keep an even strength of muscular contraction, for the subject might well relax the pressure of his grip. Since it has been shown (2, p. 85) that in normal unfatigued muscles the amplitude of the myogram varies directly with the strength

of muscular contraction, it is probable that during painful fatigue Piper's subjects did relax slightly their efforts, and this decreased the size of the action-currents. With the ergograph, however, any pull which lifts the weight calls forth a certain definite strength of muscular contraction, and since the pulling was uniformly performed, at least the beginning of any one contraction is comparable with the beginning of another. In the isotonic experiments the action-currents counted and measured were those that occurred during the upward pull on the weight; our isometric experiments are, of course, open to the same criticism as Piper's with the dynamometer.

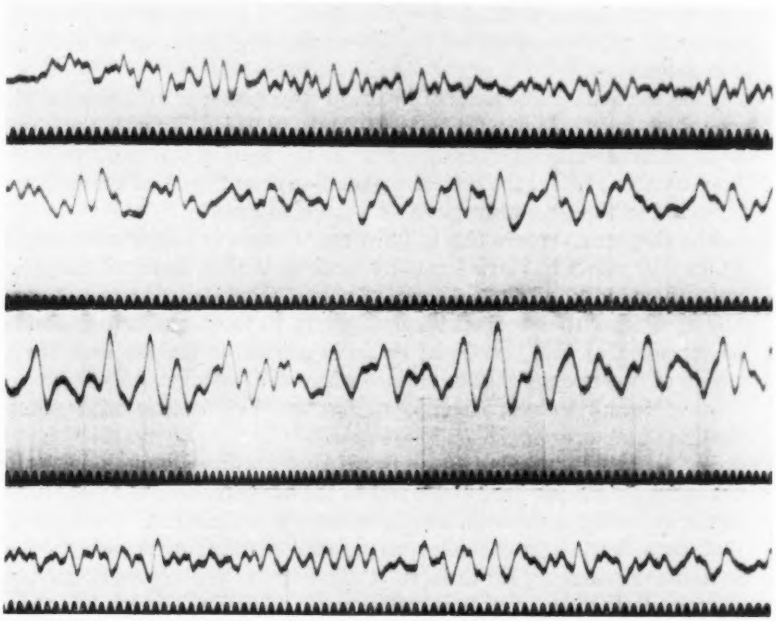
Piper's, observation that during fatigue there are more wavelets (Nebenzacken) superimposed on the main waves was not consistently corroborated by us. After counting a great many of these minute waves we decided that they were extremely variable and not of any known significance; we therefore have only counted and measured the main waves, choosing a part of the curve where they occurred rhythmically and without complication.

It might be held that these changes in frequency and amplitude of the waves were due to some technical error, but the fact that after a brief period of rest the action-currents return to normal, the technical and experimental conditions being unchanged, rules out this possibility (figs. 2C and 3D). The increase in galvanometric deflection might be due, not to a real increase in action-currents, but to a lowering of the resistance of the circuit—an effect which might easily result from the prolonged soaking of the skin under the electrodes. Therefore, careful measurements of the subject's resistance were made in some of the experiments, both before and after the work and the rest period. Although changes took place in the resistance the phenomena of fatigue did not seem to be affected, for we were able to demonstrate the increased amplitude and decreased frequency of waves as well in those cases where the resistance rose, as in those where it fell.

From table 1, which summarizes these experiments, it appears that there is a great variation in the amount of work needed to cause fatigue in the different subjects. The amount of work depends largely on the duration of the experiment, but with the ergograph used it was possible to vary the amount of work by not lifting the weight to its full height at each pull. For example, in experiment 3, S. C. did more work in 190 seconds than S. W., in experiment 7, did in 330 seconds. A comparison of the action-currents in the more quickly and more slowly fatigued subjects shows no significant difference, but all these experiments may be looked on as causing rapid fatigue.

These observations are not extensive enough to warrant any conclusions, but they indicate that it would be of interest to perform similar experiments on a large group of individuals who vary greatly in muscular

A



B

C

D

Fig. 3. Fatigue experiment with isometric contractions. (Exper. 11; June 18, 1921. See protocol.) The tuning fork marks hundredths seconds. The subject lifts "as hard as he can" against an immovable object, the electromyograms are taken from the flexor muscle:

A. At the onset of the contraction. The frequency of action-currents per second is 58, and the average amplitude of the main waves is 4.4 mm.

B. After the steady muscular contraction has been continued for 30 seconds. The frequency is 40 and the amplitude 6.5 mm. The subject complains of slight fatigue.

C. At 60 seconds, the subject complaining of painful fatigue; the frequency of action-currents is now 32 per second and the average amplitude of the waves is 9.5 mm.

D. After a rest of 60 seconds this record is taken near the onset of a new contraction, all the adjustments being just as in *C* at the end of the fatigue period. The frequency of action-currents has returned to normal, 52 per second, and the amplitude of the main waves has fallen to 5.0 mm.

strength and fatigability, for the increase in amplitude of the action-currents seems to occur most conspicuously where exhaustion is accomplished quickly. For example, A. F. (exper. 4) who accomplished 4080 centimeter-kilograms of work in 390 seconds before fatigue conquered him, showed much less variation in action-currents than T. (exper. 1) who was fatigued by 688 centimeter-kilograms of work in 150 seconds.

Theoretical. The work of Piper, so often referred to in this paper, was done before Lucas (7), (8), (9) had published his epoch-making work on neuro-muscular conduction. In the light of this work we may hope to offer a better theoretical explanation than Piper's of the changes observed in the electromyogram of a fatigued muscle.

The chapter on conduction in junctional tissues, in Lucas' monograph (9, p. 66), points out how the early work of Waller, Bernard, Langley and others indicated the existence of a special substance between nerve and muscle, and how recent investigation has accumulated evidence to suggest that this junctional tissue in a state of fatigue becomes a region of decrement—that is, a place where the propagated disturbance coming down the nerve suffers a decrease by meeting with some "resistance."

Forbes and Rappleye (10) observed that cooling a muscle without changing the temperature of the rest of the body, reduced the frequency of the muscular action-currents in voluntary contraction. Analysis of their data, in the light of the known properties of the functional response in nerve and muscle, led them to conclude that the observed frequency of muscular action currents in voluntary contraction is much less than that of the motor nerve impulses which initiate it. In dealing with the theoretical significance of our results it is necessary to have clearly in mind what is known of the character of the functional response in nerve and muscle, and especially the recovery process. The result of an effective stimulus, in either nerve or muscle, is a disturbance sweeping over the tissue from the stimulated point. This propagated disturbance seems to be of essentially the same nature whether in nerve or muscle. In either case it is marked by a change of electrical potential, such that the active portion of the fiber is negative with respect to adjacent inactive parts. In either case the disturbance obeys the "all-or-nothing" law; that is, its size is independent of the strength of stimulus, provided this be adequate. In either case the setting up of a propagated disturbance is followed by an absolute refractory period during which the tissue cannot again be excited. Following this is a relative refractory

period during which the threshold of excitation returns gradually from infinity to normal, and the size of response (as observed directly in the action-current, or indirectly by other functional criteria) returns from zero to normal. During the relative refractory phase the "all-or-nothing" law still holds good, for though the response is of subnormal magnitude, it is still independent of the strength of stimulus. The chief difference between nerve and muscle, as regards these properties, lies in the absolute duration of both response and refractory phase, being much longer in muscle than in nerve. The actual duration of the refractory period, in each case at body temperature, is roughly as follows: nerve, absolute refractory period, 0.001 second, relative r. p., 0.004 second; muscle, absolute r. p., 0.003, relative r. p., 0.004 to 0.008 second (in the cat).

Forbes and Rappleye concluded that in voluntary innervation the nerve impulses in a given motor fiber follow each other so rapidly that each comes in the relative refractory period following its predecessor, and is therefore subnormal; and for this reason the innervated muscle fiber which would respond as many as 300 times a second to a series of full-sized nerve impulses arriving with this frequency, does in fact respond to the stream of voluntary nerve impulses much less frequently, (e.g., 50 per second) since the threshold of the muscle fiber with its long refractory period remains above the stimulating value of the subnormal nerve impulses for a comparatively long time (e.g., $\frac{1}{50}$ second in the flexors of the forearm).

According to this view of the mechanism of voluntary innervation, the effect of introducing a region where conduction occurs with a decrement in the path of the disturbance, i.e., at the neuro-muscular junction, would be to reduce still further the stimulating value of the already subnormal nerve impulses, and thus prevent the muscle from responding till even later in its recovery from the refractory phase. The result of this would be twofold. It would decrease the frequency and increase the magnitude of the individual muscle responses, since, as has been indicated above, the later in the recovery process the tissue is stimulated, the larger will be its response, as well as the lower its threshold of stimulation. Thus the two effects we have observed,—decrease in frequency and increase in size of the action-currents, could both be simply explained by assuming that the effect of fatigue is merely to introduce a "resistance," or region of decremental conduction at the neuro-muscular junction.

Before assuming that this is the only possible explanation, it behooves us to consider what would be the probable effect on action-current size and frequency if fatigue were to act chiefly on the muscle fibers themselves. The work of Lapicque (11), (12) and the more recent work of Jinnaka and Azuma (13), throwing doubt on the existence of any junctional tissue, distinct from nerve and muscle, renders it especially desirable to consider the possibility of interpreting our results in terms of possible effects on the nerve and muscle fibers themselves; let us, therefore, examine the consequence of assuming that the nerve impulse arriving at the junctional point constitutes in itself the stimulus for the muscle fiber, not requiring the mediation of a junctional tissue.

In view of the well-known resistance of nerve fibers to fatigue, we may safely assume at the outset that there will be no change in the nerve impulses, traceable to this source. The only probable changes in the nerve impulses are changes in frequency, with the consequent changes in size, due to their occurrence earlier or later in the relative refractory period. Thus if fatigue caused the impulses to leave the center with a higher frequency the individual impulses would be correspondingly smaller, and having a lower stimulating value for the muscle, would excite it later in its relative refractory phase, with the same result as the introduction of a region of decrement. This view would therefore also account for both of the observed effects,—decrease in frequency and increase in the size of the muscle responses.

On the other hand, there is no positive evidence that the frequency of motor impulses is thus modified, nor any definite reason to expect such a phenomenon in the motor centers. There is, however, both in the well-known fatigability of muscle fibers, and in the localized pain felt in the muscles under the conditions of our experiments, good reason to look on the muscles themselves as the chief seat of fatigue effects. Let us then consider the effect of a rise of threshold in the muscle fibers on the frequency and size of their response. Clearly a general rise of threshold, affecting all stages of the relative refractory period, would tend to decrease the frequency of the responses, since it would delay the time when stimulation of a given strength could become effective. That is—supposing the rapid sequence of nerve impulses, constituting the source of stimulation, to remain unchanged in their stimulating efficiency—the raising of the threshold of the muscle would increase the interval after each response before the next stimulus could become available (fig. 4).

The effect of fatigue on the size of the individual muscle responses is not so easy to predict. If the resulting rise of threshold (loss of ex-

citability) is accompanied by a corresponding decrease in the size of response, the two changes affecting all stages of the relative refractory phase in the same proportion, there would be no increase in the size of

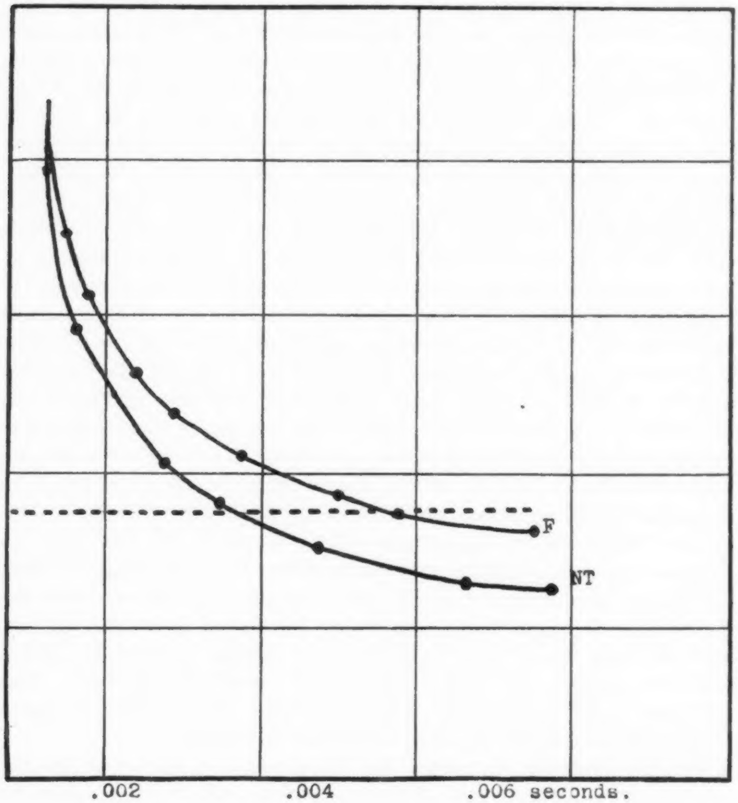


Fig. 4. *Ordinates:* Threshold in arbitrary units.

Abscissae: Time in seconds.

Curves: showing how rise of threshold due to fatigue would increase interval between responses.

Lower curve: normal recovery of threshold after response; *NT*.

Upper curve: recovery as affected by fatigue, i.e., raising of curve *NT* to position *F*.

Horizontal line: drawn at level of supposed stimulating value of nerve impulses cuts normal line earlier than fatigue line.

responses correlated with their decrease in frequency (still assuming no change in the approaching nerve impulses). For the postponement of the time of each recurring response would be offset by the diminution of the size of responses possible at any given stage of recovery. But if the effect of fatigue were to raise the threshold without causing a corresponding diminution in the size of response, then there would be correlated with a decrease in frequency, an increase in the size of the responses. Therefore to explain the observed results on the basis of fatigue effects acting on the muscle fibers themselves, without invoking the aid of change in the nerve impulses or decrement at the junction, it appears that we should be forced to conclude that if fatigue causes the threshold to be higher throughout the relative refractory phase there can be no corresponding reduction of the size of response; or at least the size of response must be less affected than the threshold.

As to the likelihood of such a selective effect of fatigue on threshold of excitation, there is not at present enough known of the nature of fatigue to justify more than a haphazard guess. It is well known that muscular activity produces acid and thus tends to increase the acidity of the immediate environment of the fibers. The relation of these changes to further activity has been discussed by Mines (14). So far as we are aware the effect of acidity on the size of action-currents has not been determined. If, as seems probable, both excitation and the action-current which marks the propagated disturbance are dependent on changes in a polarized membrane surrounding the fiber, then the effect of acid on such a membrane, in concentration sufficient to lower the excitability, would probably also reduce the size of the action-current, and we might expect the two effects to be similar in amount. But it must be granted that this supposition is speculative at best. This inference would militate against the explanation of our results as due to fatigue effects confined to the muscle fibers themselves.

We may conclude, therefore, that the decrease in frequency and the simultaneous increase in size of action-currents, which we have observed in fatigue, may be explained either by assuming a resistance or partial block to develop at the neuro-muscular junction, or by assuming that fatigue reduces the excitability of the muscle fibers without causing a corresponding reduction of the size of response of which they are capable. Finally, these observed effects might be explained as the result of an increased frequency of discharge of nerve impulses from the motor centers, but since there is no positive evidence in favor of this latter

view and much evidence tending to localize the effects in the muscle, this explanation may probably be discarded in favor of one of the other two.

SUMMARY

Electromyograms of rapidly fatigued muscles show a decrease in frequency of the action-currents, and an increase in amplitude of the individual action-currents.

It may be argued on theoretical grounds that these observations are evidence that fatigue takes place at the neuro-muscular junction, although they might be explained by fatigue acting on the muscle fibers in a selective manner, raising the threshold of excitation more than it reduces the size of action-current.

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INFLUENCE OF PANCREAS PERFUSATE ON PANCREATIC DIABETES (DOGS)[1]

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In 1916 Clark attempted to demonstrate an internal secretion of the pancreas by perfusion methods. In a series of carefully controlled experiments he first perfused the surviving heart with Locke's solution containing a known quantity of glucose and ascertained the amount of glucose utilized. He then perfused the heart and pancreas in circuit with a similar solution and found an increase in sugar utilization. Hydrolysis of the perfusate accounted for only about one-half of this increased utilization; hence he concluded that the portion unaccounted for by the hydrolysis must have been used by the heart muscle. From special experiments Clark concluded that the substance contained in the pancreas perfusate is of an enzyme-like nature.

Landes, Garrison and Moorehead perfused the surviving pancreas with a sugar-free Tyrode's solution and injected the perfusate immediately into depancreatized dogs in an attempt to delay the onset of glycosuria. The results of these experiments were in general negative although in two instances—both questionable—the glycosuria was delayed. In one experiment defibrinated diabetic blood was used as a perfusion fluid in place of Tyrode's solution. The injection of this perfusate also had no effect in delaying the onset of glycosuria.

[1] This work was completed in 1920 and the early months of 1921. The primary aim of the work was the securing of reliable evidence that the pancreas secretes anti-diabetic hormones. In the meantime Banting, Best and Macleod appear to have secured the pancreas hormones by direct extraction of the pancreas. This diminishes the importance of pancreas perfusion experiments, except in so far as they bear on the conditions under which the pancreas does or does not secrete demonstrable quantities of the hormones into the circulating medium. The present experiments are reported, as they seem to show that when the excised pancreas is perfused with blood under as nearly physiological conditions as possible secretion of the hormones into the perfusate does not take place, but toxic substances enter the perfusate, probably because of cell injury—A. J. C.

In view of these results we believed that by perfusion of the extirpated pancreas under conditions approaching more closely the physiological, and by the injection of this perfusate into diabetic dogs the course of the diabetes might be altered.

EXPERIMENTAL METHODS. Our apparatus was essentially similar to those of Clark and of Landes. It consisted of a closed system of tubes and chambers through which the perfusing fluid was circulated by means of two Woodyatt pumps. This was so constructed that it simulated the circulatory system of the animal, no bubbling or dropping of the perfusing medium occurring, so that it was essentially a closed fluid system. The perfusing medium and perfusion chamber were kept at 37°C.; oxygenation of the perfusing medium was accomplished by allowing warmed oxygen to circulate through the apparatus under a slight positive pressure, of from 1 to 3 mm. of mercury. Defibrinated blood from diabetic dogs mixed with a small percentage of glucose-free Tyrode's solution was used as the perfusing medium, it being considered the best means of carrying oxygen to the perfusing pancreas as well as fulfilling other physiological conditions. The perfusing medium during perfusion of the pancreas was kept at a pressure of from 120 to 140 mm. Hg. In all experiments where Tyrode's solution was used, we used a glucose-free Tyrode's. Sterilization of the apparatus was effected by washing with 95 per cent alcohol, which solution filled the apparatus when not in use. The elimination of bubbling and dropping of the perfusate in our apparatus was considered to be a distinct improvement over those of Clark and Landes in view of the work of Levy who demonstrated that certain enzymes, notably ptyalin, are destroyed by agitation.

Surrounding the perfusing pancreas with warmed pure oxygen was also considered to be an improvement since Bainbridge, Beddard and later Cullis found that the extirpated kidney would not secrete unless surrounded by an atmosphere of pure oxygen.

Healthy young adult dogs were used throughout. Dogs to be used for the experiments were starved 24 hours but allowed water after which time the blood sample for normal blood sugar was obtained. The animals were then depancreatinized, strict aseptic surgical technique being observed. The pancreas was completely extirpated, no trace being allowed to remain, and this fact was confirmed by autopsy. The time of operations varied from 50 minutes to 80 minutes, this being counted from the time of incision until the dog was off the table.

The dogs were kept in scrupulously clean metabolism cages. They were allowed no food, but plenty of water. The filtered urine was collected, measured and D/N ratios determined at 12-hour intervals. Blood sugar determinations of blood from the femoral vein were made, as far as possible, at time of collecting urines and at variable intervals before and after transfusion.

Urine sugar was determined by the Monson-Walker-Bertrand method and the urine nitrogen by the Kjeldahl-Gunning-Arnold method. The blood sugar was determined by the Lewis-Benedict method. All chemical determinations were made in duplicate and those that did not check within experimental limits were repeated.

In view of the more or less unsatisfactory results obtained by other workers who fed their diabetic dogs, we allowed our animals no food. Refusal or vomiting of food by diabetic dogs is liable to lead to wide daily variations in the amount of sugar excreted and this factor cannot be absolutely controlled. As a result of numerous repeated experiments we found that the D/N ratio had become almost a constant, 48 hours after operation, and usually remained near this point or grew gradually less for a period of about 7 to 10 days, unless a moribund condition intervened. In the latter event a rapid decrease was noted and in some cases no sugar was excreted for periods of 12 hours before death.

In a series of 21 diabetic dogs and with a total of 127 D/N determinations, we found 97 (or 76 per cent) of them to be between 1.2 and 2.5. In one dog (no. 16) which was running a D/N of about 2.5 for five successive 12-hour determinations, the D/N suddenly arose to 3.86. This was our highest value and cannot be accounted for except that no urine was obtained from the dog in the previous 12-hour period. With this exception and two others, none of our D/N ratios went higher than 2.9. These figures are low in comparison with those reported by Lusk but are in accordance with the general observation that the D/N ratio is decreased during starvation in diabetic animals.

The values obtained for blood sugars in a series of 25 normal dogs, 21 (or 84 per cent) were between 0.100 per cent and 0.135 per cent. In the remaining 4 dogs, (or 16 per cent) the values varied between 0.150 per cent and 0.159 per cent.

In a series of 66 blood sugar determinations in diabetic dogs our values varied between 0.245 per cent and 0.922 per cent; the latter figure was associated with a marked diminution in quantity of urine excreted and also amount of nitrogen and dextrose. In this series, 47 (or 66 per

cent) were between 0.245 per cent and 0.390 per cent, and 12 (or 18 per cent) were between 0.400 per cent and 0.595 per cent. In these determinations it was found that each dog struck an approximate level; the daily variations usually not exceeding 0.04 to 0.05 per cent. This is shown in table 1.

Dogs were not considered as suitable subjects for transfusion with pancreas perfusate until at least 96 hours following pancreatectomy, and after at least three successive D/N determinations, made at 12-

TABLE 1

Showing daily variations in blood sugar concentrations in fasting pancreatectomized dogs

DOG 21	DOG 33	DOG 37
per cent	*per cent*	*per cent*
0.361	0.247	0.301
0.370	0.259	0.308
0.380	0.278	0.297
0.401	0.253	0.318
0.389	0.290	0.391
0.371	0.285	0.297
0.309	0.252	0.311
0.321	0.261	0.323
0.382	0.246	0.310
0.348	0.252	0.301
0.326	0.277	
0.401	0.270	
	0.252	

hour intervals, had shown them to have struck a fairly constant level. At least two blood sugar determinations were always made on samples withdrawn at the time of urine collection for the above D/N's, and these values likewise were required to be within the limits of daily fluctuation as determined by experiment. When these conditions were satisfied the animals were transfused with the pancreas perfusate obtained by the following method. The pancreas of a large, healthy, young adult dog was rapidly extirpated through a long midline incision. The technique used aimed to preserve the blood supply of the organ as long as possible. The pancreas was exposed and a ligature passed beneath the pancreatico-duodenal artery, but not tied. The splenic end was then freed and the mesentery divided. The aorta was then clamped and the pancreas rapidly extirpated by dividing with Mayo scissors the pyloric end of the stomach, the duodenum at the distal

attachment of the pancreas and the pancreatico-duodenal artery and vein. The extirpated pancreas and attached bowel were immediately transferred to a pan containing N/1 salt solution at 37°C., while the pancreatico-duodenal artery was being cannulated and the bowel cut away. The organ was then suspended from a special glass rod by means of hooks and rapidly put in the perfusion apparatus, which had previously been prepared for the perfusion. *The time elapsing between the cutting off the blood supply of the pancreas and the starting of the perfusion never exceeded 4 minutes and was usually $2\frac{1}{2}$ minutes.*

Before each perfusion the apparatus was carefully washed out with distilled water, N/1 salt solution and finally Tyrode's. Seventy-five cubic centimeters of the latter were allowed to remain in the perfusion circuit. Three to four hundred cubic centimeters of defibrinated diabetic blood obtained from an animal depancreatinized at least 3 days previously were added to the Tyrode's and the whole allowed to circulate in an atmosphere of oxygen and at 37°C., while the pancreas to be perfused was being extirpated. The time that the pancreas was allowed to perfuse was varied. In one series of experiments 1 hour was allowed, in another 45 minutes, while in the third the time of perfusion was 15 minutes. Immediately following perfusion, under novocain anesthesia, 100 to 150 cc. of perfusate were slowly transfused, by means of a Woodyatt pump, into the saphenous vein of the dog under observation.

Samples for blood sugar determinations were taken from the transfused dog at intervals varying from 15 minutes to 3 hours following transfusion, and another determination was made at time of collection of first 12-hour urine sample following transfusion. All determinations were made in duplicate, as stated above.

Our first experiments were highly suggestive that a substance had been added to the perfusate by the pancreas that enabled the transfused dog to utilize carbohydrates in some manner. Dogs 1 to 6 and 13 were typical examples of this. In all of these dogs the D/N's dropped to 0.0 following transfusion and remained at practically this point for 24 hours, after which in the case of dogs 6 and 13 the glycosuria gradually returned. Dog 1 died 36 hours following transfusion and glycosuria had not reappeared at that time. Dog 6 is typical of this series and the results on him are shown in detail in table 2.

Somewhat similar results were obtained on dog 7, in which the D/N following transfusion of the pancreas perfusate dropped to less than one-half its former value in the first 12-hour sample, and the second

12-hour sample taken immediately before its death (from broncho-pneumonia) was sugar-free. This will be discussed later. No blood sugar determinations were made in these experiments.

In twelve experiments on dogs 14, 16, 18, 21, 27, 29, 31, 33, 37 and 38, the results were negative or decidedly contradictory to those obtained in the earlier work. These experiments were all controlled by blood sugar determinations, with the exception of dogs 14 and 16. Data on some of these experiments are found in table 3, while complete data on dogs 21 and 33, typical experiments of this type, are shown in tables 4 and 5. For the five 12-hour periods previous to transfusion

TABLE 2

Dog 6. Pancreatectomized January 12, 1920

TIME	URINE				REMARKS
	Amount	Total dextrose	Total nitrogen	D/N ratio	
	cc.	g.	g.		
1-17-20, 9 a.m.	305	9.49	6.26	1.52	
1-17-20, 9 p.m.	282	9.64	5.53	1.74	
1-18-20, 9 a.m.	235	7.14	4.11	1.73	
9 a.m.	Transfusion of pancreas perfusate				Pancreas perfused 1 hr.
1-18-20, 9 p.m.	230	0.19	1.92	0.10	
1-19-20, 9 a.m.	217	0.0	1.11	0.0	
1-19-20, 9 p.m.	275	0.05	4.41	1.37	
1-20-20, 9 a.m.	125	6.08	2.99	2.03	
1-21-20, 9 a.m.	73	2.68	1.43	1.87	
1-21-20, 6 a.m.					Dog died of distemper

Autopsy showed absence of pancreas fragments. Broncho-pneumonia.

of the perfusate, the blood and urine sugar, nitrogen and D/N were practically stable. Two hours and 15 minutes following transfusion the blood sugar was found to be practically unchanged. As will be noted in table 4, total dextrose and total nitrogen decreased, while the amount of urine excreted and the D/N remained practically the same. The next transfusion one day later gave practically identical results as regards blood sugar, D/N and amount of urine, but the urine dextrose and nitrogen remained almost the same. In this experiment as in many others transfusion of normal dog blood was performed and will be discussed later. In the remaining ten experiments the results obtained followed closely those given on dog 21.

A review of the data of the 12 experiments reveals the following facts: In ten cases both the urine sugar and nitrogen decreased, while of the other two experiments in one instance they both increased and

TABLE 3

Showing the influence of pancreas perfusates on diabetic dogs

TIME	URINE				BLOOD SUGAR	REMARKS
	Amount	Total dextrose	Total nitrogen	D/N ratio		
	cc.	*g.*	*g.*			
Dog 10:						
9-10-20, 9 a.m.	142	4.81	2.68	1.79		
9-10-20, 9 p.m.	300	9.42	5.25	1.79	8 p.m., 0.333%	
9-11-20, 9 a.m.	265	12.45	5.81	2.14	8:30 a.m., 0.334%	
					12:30 p.m., 0.480%	Pancreas perfused 1½ hr.
10:30 a.m.	Transfusion of pancreas perfusate					
9-11-20, 9 p.m.	255	3.24	3.88	0.84	7:30 p.m., 0.595%	
9-12-20, 9 a.m.	55	4.65	5.14	0.91	9:30 a.m., 0.816%	
10:30 a.m.	Transfusion of pancreas perfusate				12 m., 0.516%	Pancreas perfused 1 hr.
9-12-20, 9 p.m.	120	1.21	1.13	1.07	6:45 p.m., 0.644%	Dog died 9 p.m.
Autopsy: Broncho-pneumonia. No pancreas fragments						
Dog 27:						
3-21-21, 8 a.m.	315	12.85	3.58	3.59		
3-21-21, 8 p.m.	85	5.91	2.14	2.76	8 p.m., 0.301%	
3-22-21, 8 a.m.	210	13.82	5.92	2.33	7:45 a.m., 0.308%	
					2 p.m. 0.297%	Pancreas perfused 1 hr.
10:30 a.m.	Transfusion of pancreas perfusate					
3-22-21, 8 p.m.	120	5.52	2.16	2.56	8 p.m. 0.318%	
3-23-21, 8 a.m.	105	2.89	1.85	1.56	8:30 a.m. 0.331%	
10:30 a.m.	Transfusion with 165 cc. defibrinated dog's blood				4 p.m. 0.297%	
3-23-21, 8 p.m.	175	6.90	5.20	1.32	7:45 p.m., 0.311%	
3-24-21, 8 a.m.	90	1.66	1.63	1.02	8 a.m. 0.323%	
9:33 a.m.	Transfusion with 150 cc. defibrinated dog's blood				9:53 a.m., 0.310%	
3-24-21, 8 p.m.	280	4.65	3.18	1.45	8:15 p.m., 0.301%	

Dog killed and found free from pancreas fragments.

in the other they both remained the same. In four out of the twelve experiments the D/N's remained the same, showing that the decrease in dextrose and nitrogen was relatively equal. In five experiments increases of approximately 10 to 20 per cent in the D/N's were obtained, and in four of these cases the urine nitrogen dropped lower, out of

TABLE 4

Complete data on the influence of pancreas perfusate on diabetic Dog 21. Pancreatectomy performed September 5, 1920

TIME	URINE				BLOOD SUGAR	REMARKS
	Amount	Total dextrose	Total nitrogen	D/N ratio		
	cc.	*g.*	*g.*			
9-9-20, 9 a.m.	138	5.28	2.64	1.99		
9-9-20, 9 p.m.	140	7.48	3.75	1.99		
9-10-20, 9 a.m.	205	8.57	4.22	2.03		
9-10-20, 9 p.m.	210	7.56	3.97	1.90	8 p.m. 0.361%	
9-11-20, 9 a.m.	160	7.41	3.58	2.07	8:30 p.m., 0.570%	
10:30 a.m.	Transfusion with pancreas perfusate				12:45 p.m., 0.380%	Pancreas perfused 1½ hrs.
9-11-20, 9 p.m.	163	4.47	2.06	2.12	7:30 p.m. 0.401%	
9-12-20, 9 a.m.	260	5.30	2.96	1.79	9 a.m. 0.389%	
					12:30 p.m. 0.371%	Pancreas perfused 1 hr.
10 a.m.	Transfusion with pancreas perfusate					
9-12-20, 9 p.m.	245	5.29	2.80	1.89	0.309%	
9-13-20, 9 a.m.	150	6.35	3.47	1.80	10 a.m. 0.321%	
9-13-20, 9 p.m.	100	3.53	2.10	1.68	8:30 a.m. 0.382%	
9-14-20	150	5.98	3.53	1.69		
10 a.m.	Transfusion with 150 cc. defibrinated dog's blood				1 p.m. 0.348%	
9-14-20, 9 p.m.	105	2.35	1.33	1.77	8 p.m. 0.326%	
9-15-20, 9 a.m.	190	4.43	3.28	1.35	8:45 a.m. 0.401%	Dog abandoned here

Dog killed and found free from pancreas fragments

proportion to the fall in dextrose. The results of blood sugar determinations on ten experiments showed no relationship to the sugar or nitrogen excretion or D/N ratio. In eight cases the value increased or decreased slightly but remained within limits that can be considered normal daily variation. In the other two cases, both in same dog (no. 18),

one result was exactly opposite the other. In the first instance there was a marked retention of sugar and nitrogen and the blood sugar value rose from 0.334 per cent in a sample just previous to transfusion to 0.48 per cent 2 hours following transfusion and to 0.595 per cent 9 hours after transfusion. The urine sugar and nitrogen were steadily decreasing during this period. In the second instance both urine sugar

TABLE 5

Complete data on dog 33. Complete pancreatectomy September 18, 1920

TIME	URINE				BLOOD SUGAR	REMARKS
	Amount	Total dextrose	Total nitrogen	D/N ratio		
	cc.	*g.*	*g.*			
9-20-20, 8 a.m.	250	3.96	2.52	1.56		
9-20-20, 8 p.m.	205	4.88	3.18	1.53	7:55 p.m., 0.247%	
9-21-20, 8 a.m.	165	5.51	2.61	2.11	7:40 a.m., 0.259%	
9:40 a.m.	Transfusion with pancreas perfusate				11.15 a.m., 0.278%	Pancreas perfused ¼ hr.
9-21-20, 8 p.m.	63	2.90	1.30	2.23	8:30 p.m., 0.253%	
9-22-20, 8 a.m.	220	3.69	2.65	1.39	8:25 a.m., 0.290%	
9-22-20, 10 p.m.	Transfusion with spleen perfusate				10:30 a.m. 0.285%	Spleen perfused ¼ hr.
9-22-20, 8 p.m.	55	1.75	1.40	1.25	8:10 p.m. 0.252%	
9-23-20	225	1.65	2.40	0.69	7:30 a.m. 0.261%	
9:30 a.m.	Transfusion with 150 cc. defibrinated dog's blood				10:10 a.m. 0.245%	
9-23-20, 8 p.m.	110	2.01	1.68	1.20	7:45 p.m. 0.252%	
9-24-20, 8 a.m.	120	2.88	2.23	1.28	7:25 a.m. 0.277%	
8:45 a.m.	Transfusion with 150 cc. defibrinated dog's blood				10:05 a.m. 0.270%	
9-24-20, 8 p.m.	180	3.24	2.54	1.28	8:15 p.m. 0.252%	
9-25-20	110	0.90	2.03	0.44		

Dog killed and found free from pancreas fragments

and nitrogen rose following transfusion and the blood sugar value dropped from 0.816 per cent just previous to transfusion to 0.516 per cent, $1\frac{1}{2}$ hours after transfusion and rose again to 0.644 per cent, $6\frac{3}{4}$ hours later.

Earlier workers have generally considered that the decrease in the amount of dextrose excreted after various experimental procedures

indicates that the carbohydrate had been utilized by the diabetic animals. Later workers, notably Lusk, pointed out the fallacy of this assumption. In practically all our experiments we observed marked decreases in urine dextrose following transfusion of the pancreas perfusate; but we found also that the urine nitrogen decreased and usually proportionately to the fall in sugar, so that in practically every instance the D/N value, which Lusk has shown to be the true criterion of carbohydrate metabolism in diabetes, remained practically the same. In all these experiments, except the case noted below, dog 18, the blood sugar values remained practically the same or within the limits of daily variation. In the two cases where the D/N decreased, the first, (dog 16), the D/N value immediately before transfusion of the perfusate was 2.62 and 12 hours later it dropped slightly to 2.11. As will be seen from these values it was still too high to be considered positive evidence. Unfortunately no blood sugar determinations were made on this dog. In the second case (dog 18) the D/N decreased from 2.14 to 0.83 following transfusion and the urine sugar from 12.45 grams to 3.24 grams and the nitrogen from 5.815 grams to 3.876 grams and 12 hours later there was further diminution in both urine sugar and nitrogen. It is interesting to note the course of the blood sugar determinations in this experiment. Two hours following transfusion the value obtained was 0.480 per cent, having risen from 0.334 per cent. Seven hours later it had reached 0.595 per cent and continued an upward course in almost inverse proportion to the progressive decrease in urinary dextrose. This result seems to show that the sugar was retained in the blood and not being excreted as indicated by the marked decrease in the urine sugar and D/N ratio. It is quite likely that this same phenomenon occurred in our first experiments which were not controlled by blood sugar determinations.

In order to ascertain whether our results with the transfusion of pancreas perfusate were peculiar to perfusates of that organ, a control experiment was carried out using the spleen instead of the pancreas. In this experiment (dog 33) the results of which are shown complete in table 5 above, the urine sugar, nitrogen and D/N as well as the blood sugar values all behaved similar to 90 per cent of the experiments in which the pancreatic perfusates were used. The urine sugar just before transfusion was 3.69 grams, while the nitrogen was 2.65 grams and the D/N 1.39. Twelve hours after transfusion with the splenic perfusate the sugar and nitrogen had decreased to 1.747 grams and 1.403 grams respectively, while the D/N diminished slightly from

1.39 to 1.25, the latter value being well within the limits of daily fluctuation. The blood sugar before transfusion was 0.290 per cent and and $1\frac{1}{4}$ hours after transfusion 0.285 per cent, and $9\frac{1}{2}$ hours later 0.252 per cent. Hence, it will be seen that the splenic perfusate and pancreas perfusate as judged by these tests had the same effect when transfused into the diabetic animal.

We next desired to find out if normal defibrinated blood had any effect on the D/N, urine sugar and nitrogen, or blood sugar of the diabetic animal since we used defibrinated diabetic blood in our perfusing medium. Under ether anesthesia blood was taken from the carotid artery of a normal dog and defibrinated. One hundred and fifty cubic centimeters of this were slowly injected into the saphenous vein of a diabetic dog, the vein being isolated under novocain anesthesia. Eight experiments were performed in this series, some of the results of which are shown in tables 3, 4, and 5 above. In these experiments the D/N and blood sugars were the most constant factors. Both remained practically the same despite marked irregularity in the values obtained for urine sugar and nitrogen. These results are quite at variance with those found by other workers, notably Carlson and Drennan, who found consistent decrease in dextrose excretion following the transfusion of normal blood into diabetic animals.

For reasons similar to the above we performed an experiment to determine the effect of N/10 salt solution when transfused into the diabetic animal. In this experiment, D/N, urine sugar and nitrogen, as well as the amount of urine excreted, were markedly increased. However, the blood sugar values remained unchanged. Hence, we concluded that salt solution at least had no inhibiting effect on the glycosuria.

In view of our negative results with the transfusion of pancreatic perfusates it was deemed advisable to further control our work. So at the suggestion of Doctor Carlson we attempted to obtain the external secretion from the perfused pancreas, using the same procedure as we had heretofore used in similar perfusions except that the duct of Santerini was cannulated before the pancreas was extirpated. These experiments were considered to be a possible measure of the viability of the extirpated pancreas, it being held that if the external secretion could be obtained from an extirpated pancreas under perfusion methods as above this would be a strong argument in favor of the physiological state of the pancreas.

A preliminary experiment was performed in which its normal flow and response to secretion were observed in situ on a dog anesthetized

with ether. The blood supply to the organ was then completely cut off by means of hemostats and the pancreas became very cyanotic. The pancreas was subjected to this temporary ischemia for periods varying from 1 to 12 minutes, after each period the hemostats being removed and the blood again allowed to flow through and the response to secretin noted. It was found that after periods of ischemia as long as 12 minutes the organ still responded to secretin injected into the femoral vein. Hustin reports a number of experiments in which he perfused the extirpated pancreas with blood and Locke's solution containing secretin and he obtained a flow of pancreatic juice. The amount of secretion, however, was very small and it is doubtful if it represents any more than the preformed amount of juice remaining in the pancreatic ducts at the moment of extirpation. His controls, particularly those in which he used blood alone, are not above question. We are continuing the work of perfusion of the extirpated pancreas from the standpoint of external secretion.

From a review of all of our experiments we believe that the injection of pancreas perfusates or normal blood into the blood circulation of a diabetic dog does not result in utilization (glycogenesis or oxidation) of dextrose by the animal. The evidence lies first in the fact that in all experiments controlled by blood sugar determinations the hyperglycemia was usually unaffected. Secondly, the D/N values obtained remain fairly constant or increased following transfusion and in those three cases in which blood sugar values were determined and the D/N decreased the diminution was not sufficient to ascribe it to utilization of dextrose, since the amount of dextrose in the urine was still considerable. Of our three early experiments, uncontrolled by blood sugar determinations, in which the glycosuria entirely disappeared following transfusion of a pancreas perfusate, the first (dog 1) rapidly developed a moribund condition following transfusion. It is a well-established fact that glycosuria diminishes or disappears just previous to death, so that this result may be questioned. This explanation cannot be applied to the other two experiments for in these glycosuria reappeared 24 hours after its disappearance following transfusion of the perfusate. The results of these two experiments we cannot explain. The fact that the transfusion of a splenic perfusate gave results similar to those found on injection of pancreas perfusate indicates that the action of perfusates of the latter organs are not specific.

In our experiments with the transfusion of normal blood into the diabetic animal we found that the blood sugar values were markedly

constant and the D/N ratio in practically every case was essentially unaffected. Furthermore, we did not find the marked and constant decreases in glycosuria noted by other workers.

Landes, Garrison and Moorehead found that transfusion of a pancreas perfusate into a diabetic animal gave a diminution in the amount of sugar and nitrogen excreted but that the D/N remained almost the same. They used Tyrode's solution as the perfusing medium. We obtained similar results using blood as the perfusing fluid. To eliminate blood as a possible source and to check other workers, we transfused normal blood and the results obtained did not show the consistent decreases in urine sugar and nitrogen as found in the experiments in which pancreas perfusate was used or as noted by Drennan and others.

Kleiner has recently reported a number of experiments in which diabetic dogs were injected slowly with neutral or slightly acid emulsions of dog's pancreas. A marked reduction of blood sugar concentration and glycosuria was noted in practically every case.

Similar decreases in blood sugar concentrations were not noted in control experiments in which submaxillary gland, spleen and muscle were used, although in a few instances decreases in glycosuria were observed. The D/N ratios were not determined, so it was not known whether sugar was actually utilized. Furthermore, the fact that these organ emulsions had stood in a refrigerator for from 1 to 20 hours before being injected might lead us to suspect that the decrease in glycosuria was due to depressor substances, resulting in a temporary diminution in excretion of urine and thus urine sugar. Moreover, in view of the work of McGuigan, who showed a marked hypoglycemia resulting from injections of peptone solutions with consequent drop in blood pressure, we feel that Kleiner should have controlled his experiments by taking continuous blood pressure tracings. Apparently Kleiner was not familiar with the work of McGuigan. Although his controls of submaxillary gland, spleen and muscle did not show the marked decreases in blood sugar values found with pancreas emulsions, it is well known that none of the above substances contain the powerful enzymes present in the pancreas. We believe he should have tried the injection of pancreas emulsions the enzymes of which had been inactivated by heat. Our results appear to be more similar to those of Alexander and Ehrman and of Hess, who found no effect or only a temporary decrease in glycosuria following transfusion of normal

blood into diabetic dogs; and also of Lepine, who found in similar experiments the blood sugar concentration to be unaltered.

Furthermore, the results obtained with a perfusate of the isolated spleen were similar to those obtained with the pancreas perfusate.

Our evidence points to organ perfusate as containing something that markedly affects the excretion of sugar and nitrogen. How is this accomplished? The first possibility is polymerization or oxidation of the dextrose in the diabetic animal. If this had occurred we should expect a diminution in the sugar excreted but an increase, or at least, the same amount of nitrogen to be present. Thus the D/N should be decreased consistently. In our experiments both the sugar and nitrogen excreted markedly decreased following transfusion of the perfusate leaving the D/N approximately the same. Hence, we conclude that polymerization or oxidation could not have occurred.

Secondly, if the effect had been on the kidney, depressor or toxic, and thus have prevented sugar excretion we should expect an increase in hyperglycemia. But our blood sugar values remained unchanged. Hence, it seems improbable that the kidney is the principal factor concerned. Can it be, however, that there is a mechanism preventing the hyperglycemia rising above a certain point in the individual diabetic as in the normal?

Pancreas perfusates have been shown by other workers to have a depressor action.

There is a possibility that the pancreas perfusate acts directly on the body cells diminishing the rate at which protein is broken down.

SUMMARY

1. Pancreatic perfusates, obtained by perfusing (15 to 60 minutes) the excised dog pancreas with defibrinated diabetic dog's blood slightly diluted with Tyrode's solution, transfused into completely pancreatectomized dogs, do not consistently reduce the hyperglycemia and glycosuria. There may be a temporary decrease in the excretion both of sugar and nitrogen in the urine. Similar results are secured by transfusion of spleen perfusates into diabetic dogs.

2. Perfusing such organs as the pancreas or the spleen, even under the closest approximation to physiological conditions, appears to add toxic substances to the perfusate, and as judged by the extremely slight response of the perfused pancreas to secretin the cells are probably not in condition to secrete the hormones. Under more artificial

conditions of perfusion, such as the use of pure Ringer or Tyrode solutions, and prolonging the perfusing time, the hormones may enter the perfusate, not by cell secretion, but by cell extraction or solution. Such perfusates become essentially crude organ extracts, containing also substances that are toxic on intravenous injection.

We wish to record our appreciation of the valuable aid and many helpful suggestions given us by Dr. A. J. Carlson, under whose direction this work was carried out. We are also indebted to Dr. F. C. Koch of the department of Physiological Chemistry for his kindly aid and advice.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

V. The Hepatic Factor in Chloroform and Phosphorus Poisoning

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Our knowledge of the functions of the liver has been obtained mainly from three general sources: *a*, acute physiologic experiments on the organ; *b*, studies of disease conditions of the liver; and *c*, the effect of hepatectomy. The observations concerning the functions of the liver which are most emphasized clinically are those which have been made in connection with the diseases of the organ. This method of studying liver function was opened to experimental investigation when it was noted that certain poisons, notably chloroform and phosphorus, seemed more or less specifically to injure the liver. Many experiments have been performed by various investigators for the purpose of determining the effect of these poisons, and owing to the fact that the liver is greatly injured by the poisons, most of the changes observed have been ascribed to damaged liver function. As a corollary to this the liver has been credited with those physiologic activities which were found to be damaged by the poisons. Many of the alleged functions of the liver have been assumed to be proved by this method of investigation.

Our numerous experiments on the effect of total removal of the liver have clearly proved that this organ has a vital function with regard to the maintenance of a definite blood-sugar level (4), (5), (6). Although the liver has many functions, several of which may be vital, the cause of death following the total loss of its function is primarily a failure to maintain the proper blood-sugar level. This definitely proved fact gives a test for the total absence of hepatic function, and a method of determining, in a fairly definite manner, whether chloroform and phosphorus do specifically destroy the function of the liver. Therefore, our purpose in making the experiments reported here was to determine whether the same effect is produced when the liver is supposedly destroyed chemically with chloroform and phosphorus as when it is totally

removed surgically. When the liver is totally removed surgically, three definite things are observed: *a*, the blood sugar decreases; *b*, associated with this hypoglycemia, a characteristic group of symptoms develops; and *c*, these symptoms are relieved when glucose is administered. If this sequence of events occurred after a fatal dose of the poisons in the same manner as after surgical removal of the liver, it would be conclusive proof that the cause of death following administration of chloroform and phosphorus was due to insufficiency of the liver. It would, furthermore, greatly strengthen the belief that the results produced by the poisons are dependent wholly on damaged hepatic function, and would substantiate the many alleged functions of the liver, the assumption of which is based on the action of these poisons. The data thus obtained would give us a criterion for the proper comparison of the results obtained by chemical destruction of the liver, and the alleged functions based on these results, with those we have obtained by total surgical removal of the liver.

The normal dog does not have uric acid, or only a trace in the blood, but after total removal of the liver a considerable amount of this substance is usually found. We are not prepared definitely to assert that the accumulation of uric acid in the blood is directly owing to the loss of function of the liver, but our observations tend to substantiate such a conclusion and also to show that this particular function is the most easily impaired of any we have studied. For this reason we have also noted the presence or absence of the uric acid in the blood. We thus have in the characteristic symptoms and hypoglycemia a definitely proved test for total loss of function of the liver and, in the appearance of uric acid in the blood, a very suggestive test for slight hepatic damage.

Incidentally, we have studied many of the other constituents of the blood, including urea, protein and non-protein nitrogen and creatinin, and have also studied the function of the kidney with phenolsulphonephthalein. These observations, however, are considered secondary to those on the blood sugar and uric acid.

While a large number of investigations have been made on various phases of chloroform and phosphorus poisoning, very little work has been done on the effect of such poisons on the blood sugar, or carbohydrate metabolism. Frank and Isaac (3) made blood-sugar determinations on rabbits which had been poisoned by large doses of phosphorus. These authors concluded from a rather large series of experiments that there was a terminal hypoglycemia. Opie and Alford (8), in feeding experiments, have found that the toxicity of chloroform and phosphorus

to the liver can be influenced by the character of the diet. They found that the injury to the hepatic tissue is least for each unit of poison if the animal is on a diet rich in carbohydrate, and greatest on a protein diet. Neubauer (7) made glycogen determinations of the liver and muscles of animals poisoned with phosphorus, and found that there was in general a decrease in the amount of glycogen present.

METHOD OF INVESTIGATION. Our experiments were performed on normal, healthy dogs. The blood sugar and uric acid were estimated before the experiments were begun and at various periods following the administration of the poison. Samples of the blood were taken from the external jugular vein. The Benedict (1) modification of the Lewis-Benedict method was used for blood-sugar estimation, and the Folin (2) method for uric acid determination was employed in all experiments. In most of the experiments a definite time relation was maintained between the feeding of the animal and the procuring of the blood sample.

The routine technic for obtaining data on the animals given chloroform consisted of withholding food for a definite length of time in some instances, and in others, not withholding it. Before the induction of anesthesia, blood was obtained for sugar and uric acid determinations as well as for the other blood constituents when they were included. The animal was then placed in an etherizing cabinet and anesthetized. When under the influence of the ether the animal was removed from the cabinet, placed on the operating table, intubated and anesthetized by chloroform intratracheally. The anesthesia was counted as beginning with the administration of the chloroform. At the conclusion of the anesthesia, another sample of blood for sugar and uric acid estimation was taken, and samples were taken twice daily until the conclusion of the experiment.

The experiments with phosphorus differed from those with chloroform in several details, but in essentials they were the same. The animals that were given phosphorus were not deprived of food before the experiment. Blood was taken before the first administration of phosphorus and twice daily thereafter. Phosphorus was given by mouth in an emulsion of cod liver oil in capsules, each capsule containing 0.05 mgm. of phosphorus. In some experiments two capsules were given daily and in others only one.

EXPERIMENTS WITH CHLOROFORM. Chloroform was administered in forty-five experiments on twenty-six normal dogs and the blood sugar and uric acid and occasionally other constituents in the blood

were carefully noted. In thirteen of the experiments the animals had been deprived of food for a period of time varying from 24 to 96 hours. Food was not withheld in nine experiments, while in the remainder the fast of 16 hours used routinely in all experiments in the laboratory was employed. The time during which the animal was kept under chloroform anesthesia varied from 1 to 7 hours. In some experiments the chloroform was administered two or more times with varying intervals between; in one instance it was administered six times. The normal blood-sugar values in the entire series varied from 0.15 to 0.07 per cent. This is a greater variation than is usually observed and is probably dependent on the fact that the specimens of the blood were necessarily obtained at various periods of time in relation to the ingestion of food and to fasting. Uric acid was not found in the specimens of normal blood. The blood-sugar content immediately after withdrawal of the chloroform was usually higher than before anesthesia; the rise was often very slight, but in a few instances the content was very high. The blood-sugar content remained normal or above normal throughout twenty-four of the experiments. In fifteen of these experiments the animals recovered, and in nine they died; five died during or very soon after the close of the anesthesia; two died from the effects of the chloroform 48 hours after the anesthesia, and two died at the end of 96 hours.

In fifteen of the experiments there was slight decrease in blood sugar, although it remained within normal limits and at no time decreased below 0.065 per cent. This decrease usually occurred 24 to 48 hours after administration. Only two of the animals in this group died from the effects of the chloroform, death occurring 48 hours after the administration.

The remaining six experiments were the only ones in the series in which there was a significant decrease in the blood sugar which compared in any way with the decrease following hepatectomy. Two of the animals recovered, and two died before the end of 24 hours. One died 36 hours and the other 72 hours after completion of the anesthesia. The blood-sugar estimates were 0.065, 0.06, 0.053, 0.049, 0.05 and 0.058 per cent respectively at the time of death.

All the animals were observed carefully for symptoms which might simulate those noted in relation to the hypoglycemia following hepatectomy. It was not possible, however, owing to the length of the experiments, to keep all the animals under constant observation. Fifteen of the animals died from the effects of the chloroform; five of these died at various periods after the completion of the anesthesia

and were observed almost constantly until termination of the experiment. In none of the animals were symptoms observed which corresponded to those associated with hepatectomy. Two of the animals developed convulsions and other symptoms associated with salivation. These symptoms were not the same as those noted after total removal of the liver. In both animals, the blood sugar was above normal at the time of the convulsions. One of the animals was benefited by intravenous injection of glucose; the other was not helped by glucose but was slightly benefited by the intravenous administration of a 10 per cent solution of sodium chlorid.

Uric acid in the blood was noted more constantly than changes in the blood sugar after the administration of chloroform. In sixteen of the experiments a very appreciable amount of uric acid appeared, varying from 1 to 5 mgm. for each 100 cc. of blood; in eleven of these the animals died from the effects of the chloroform. In twelve experiments a trace of uric acid appeared; in two of these the animals died. In the remaining seventeen experiments uric acid did not appear in the blood, and in two of these the animals succumbed to the chloroform.

Experiments with phosphorus. Phosphorus was administered to eight animals until they succumbed. In three of the animals there was marked terminal hypoglycemia; in the others the blood sugar remained within normal limits throughout the experiment. In no instance were symptoms simulating those noted after hepatectomy observed. Seven of the animals had uric acid in the blood, in six of which the amounts were considerable.

Experiments with chloroform and phosphorus after pancreatectomy. Our previous experiments have proved that hepatectomy produces a marked decrease in the condition of hyperglycemia following pancreatectomy. In one series of experiments pancreatectomy was performed 24, 48 and 72 hours, respectively, before administration of the chloroform. The blood sugar in each instance increased as usual following pancreatectomy. The three animals died within the first 24 hours after the administration of chloroform. In two of the experiments the blood sugar decreased markedly within the last few hours of life; in one experiment it increased progressively after the administration of chloroform. Uric acid appeared in the blood of each animal. In four experiments phosphorus was administered, beginning 48 hours after pancreatectomy. In two of these, marked terminal decrease in the blood sugar was noted. Uric acid appeared in the blood of each animal.

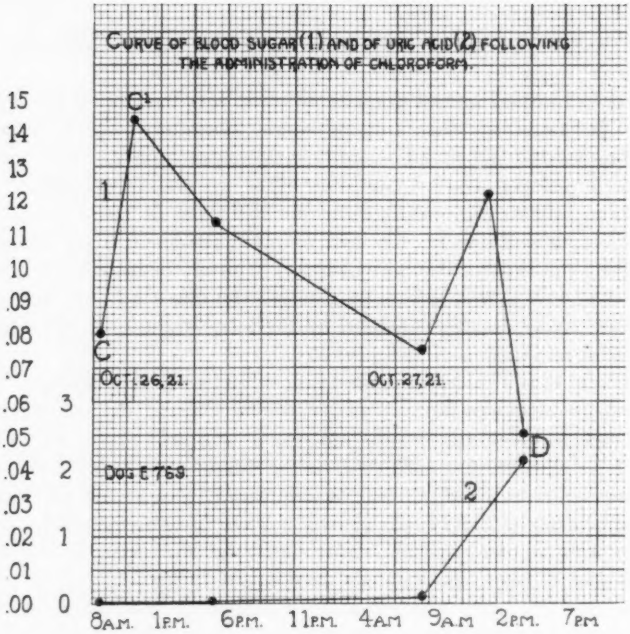

Fig. 1. In the experiment illustrated by this chart the effect of administration of chloroform on blood sugar and uric acid in the blood more nearly simulated the effect of hepatectomy on the same constituents of the blood than any of the other experiments following the use of this drug. The chloroform was administered during the period marked *C* and *C-1*, and at *D* the animal died. Note the terminal decrease in blood sugar and accumulation of uric acid in the blood.

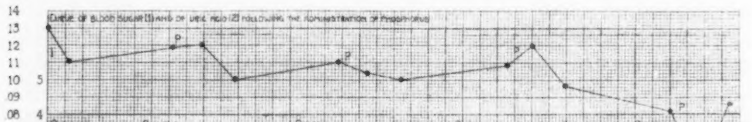

Fig. 2. This chart illustrates a terminal decrease in blood sugar and accumulation of uric acid in the blood following the administration of phosphorus. Phosphorus was administered at points marked *P*, and at *D* the animal died. The curves here, as well as those shown in Figure 1, would signify profound damage to the liver produced by these drugs, and if these results had been uniform it would have seemed that a more or less complete functional removal of the liver by chemical means would be possible.

Discussion. The results of these experiments were variable and in many respects unsatisfactory. There was such a wide difference in the reaction of the animals to the poisons that we were never able to produce a definite result by a definite dosage. Certain animals died after 2 hours of chloroform anesthesia, whereas one survived six periods. In the latter experiment, a few days intervened between the periods

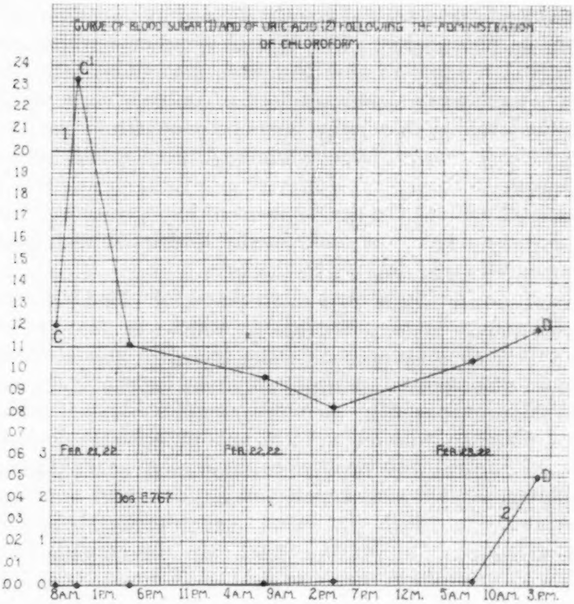

Fig. 3. In the experiment illustrated by this chart, the animal died from the effects of the administration of chloroform, but hypoglycemia did not develop, although uric acid accumulated in the blood shortly before death. The chloroform was administered at the points marked *C* and *C-1* and the animal died at *D*. This has been a more constant finding than those shown in Figures 1 and 2.

of anesthesia, and the periods were $2\frac{1}{2}$ hours, 3 hours, 3 hours, 4 hours, 5 hours and 5 hours, respectively, making a total of $22\frac{1}{2}$ hours. Several of the animals which succumbed to the chloroform died so soon after its administration that the experiments were not of much value. This was particularly true when two or more periods of anesthesia were necessary. As the length of life after administration was variable, we

were not always able to observe the terminal symptoms, even though the animals were kept under hourly observation during the day and were seen two or three times during the night. An animal which appeared to be in fair condition at midnight, might be found dead at 6 o'clock in the morning.

The effect of the poisons on the blood-sugar content was variable. In most instances, except for the initial hyperglycemia from the anesthetic, the blood sugar was not greatly affected by the administration of chloroform or phosphorus. The typical symptoms which invariably follow surgical removal of the liver were never observed. In a few experiments there was a significant decrease in blood sugar; this seemed to be terminal, taking place a few hours before death. The few experiments in which a marked hypoglycemia occurred would indicate very serious damage to the liver. However, in several experiments the outcome was fatal without the development of hypoglycemia. The death of these latter animals was undoubtedly associated with some cause other than physiologic damage to the liver.

The change in the blood most often noted following the administration of chloroform and phosphorus, which is also noted after hepatectomy, is the appearance of uric acid. In animals from which the liver has been removed surgically, uric acid invariably appears in the blood quickly, and usually increases considerably. Animals which have been poisoned with chloroform and phosphorus do not always have uric acid in the blood, and it does not develop so rapidly, or in such large amounts, as following hepatectomy. Uric acid appeared in all the experiments in which the blood sugar decreased, and in many instances in which it remained normal. In the animals which were poisoned with chloroform, the uric acid, if it appeared at all, did so within 20 to 48 hours after the anesthesia. If the result was not fatal, the uric acid disappeared within 12 to 24 hours.

We do not feel justified at the present time in definitely ascribing the development of uric acid in the blood primarily to damage to the function of the liver. However, all our observations would tend to substantiate such a conclusion, and further would seem to show that the function of the liver in relation to purin metabolism is the most easily impaired of any which we have studied. However, if the development of uric acid is a test of impaired liver function, it would, in view of our results with chloroform and phosphorus, demonstrate two points: *a*, since it did not appear in all the animals, not even in some which died from the effect of the poison, it would appear that damage to the liver was not respon-

sible for death in these instances; and *b*, it did appear in most of the animals, and if they recovered, it disappeared at about the period after the chloroform anesthesia which Whipple (9) has shown to be coincident with the beginning of histologic repair of the liver.

In approximately half of the experiments, estimations of urea in the blood were made. In most of these no change in the urea content was noted; this was particularly true in experiments in which the dose was not fatal. In some of the animals a marked terminal increase in urea was noted; this has not been observed following surgical hepatectomy.

The small number of experiments in which there was profound hypoglycemia, associated with an accumulation of uric acid in the blood, would seem to show definitely a damage to the functions of the liver by the poisons, even though the characteristic symptoms did not develop. The slight and usually transient decrease in blood sugar and the transient appearance of uric acid in the blood noted in certain other experiments in the series would also seem to signify physiologic damage to the liver. However, the very definite association of symptoms and blood findings which occurs when the liver is totally removed by surgery does not seem to occur following administration of chloroform and phosphorus. This shows that the liver is not made totally functionless, and that other factors are at least partially responsible for death.

Our experiments, while emphasizing the importance of physiologic damage to the liver, definitely show that such damage is not the only factor, and in many instances probably not the primary factor, in death following the administration of chloroform and phosphorus. The process involved is complex and not only includes the effect of the poison on other organs such as the heart, kidneys and adrenals, but also the effect on the general physiologic activity of the organism of damage to, or destruction of a large amount of body tissue. It has seemed to us that the products of cell disintegration which result from the action of chloroform and phosphorus must be a potent factor in the cause of death. This would seem to be particularly true in those experiments in which the blood urea was high, the blood sugar normal, and the phenolsulphonephthalein output of the kidneys normal. The effects of the poisons are so complicated that definite conclusions apparently cannot be drawn with regard to the function of any one organ, as for instance the liver, even though this organ seems to be, and probably in most instances is, the most seriously damaged histologically and physiologically.

SUMMARY

A series of experiments was performed to determine whether the characteristic symptoms and the concomitant changes in the blood, namely, a decrease in blood sugar and the appearance of uric acid, which invariably follow total removal of the liver by surgery, would also occur after the administration of chloroform and phosphorus. The characteristic symptoms which follow hepatectomy were not observed after poisoning with chloroform or phosphorus, although the results of the poisoning were in many instances fatal. Hypoglycemia occurred in but a small percentage of these experiments and uric acid in the majority.

The results conclusively prove that the poisons, while greatly injuring the functions of the liver, do not produce complete hepatic insufficiency except possibly in a very small number of instances. Other tissues and organs are undoubtedly profoundly affected, and in many instances the functional damage to the liver is probably not the primary cause of death. However valuable studies on the effect of chloroform, phosphorus and allied poisons may be, particularly in relation to hepatic disease, conclusions with regard to the function of the liver based on such studies must be very cautiously drawn.

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THE ALLEGED EFFECTS ON BODY GROWTH AND GONAD DEVELOPMENT OF FEEDING PITUITARY GLAND SUBSTANCE TO NORMAL WHITE RATS

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The following feeding experiments were undertaken at the suggestion of Doctor Carlson, because of the contradictory data in scientific and clinical literature concerning the influence of feeding pituitary substance.

Sandri, Schafer, Aldrich, Lewis and Miller, Klinger, Sisson and Broyles report essentially no effects on body growth and gonad activity from feeding and injection of hypophysis substance or implantation of the living hypophysis in dogs, rats and guinea pigs. Robertson, Goetsch, and Marinus report some stimulation of body growth and gonad activity by pituitary feeding in the rat. Uhlenhuth, Smith, Hoskins and Hoskins report some stimulation of growth from hypophysis feeding in amphibian larvae. Robertson fed anterior lobe substance to mice and reported a primary retardation followed by stimulation of growth. Goetsch, and Marinus reported acceleration of growth and gonad activity on feeding anterior lobe to white rats. Marinus used fresh glands in his feeding experiments. The data concerning the influence of pituitary gland feeding to chickens are equally contradictory, Clark reporting a stimulation of the ovaries (increased egg laying) while Pearl and Surface deny it. The clinical literature is not only contradictory, but the apparent positive results are even more difficult to interpret because of the lack of controls.

Experimental methods. Two series of feeding tests were run, each series containing eight litters of rats. The feedings were started when the rats were 4 weeks old, and weighing 30 to 40 grams. Each litter was divided so as to have both males and females in each group. In the first series the feedings were continued for 16 weeks. In this series the main factor studied was body growth, and for that purpose the rats were weighed once each week. In the second series we desired to study

the rate of maturation of the gonads. In this series the rats were fed for 4 weeks, killed by ether, and ovaries, uterus and testes weighed and sectioned for microscopic studies.

The pituitary gland material (anterior lobe, posterior lobe, whole pituitary) used was Armour's desiccated pituitary substances. As controls we fed equal quantities of desiccated brain substance. The quantities of pituitary substances and brain fed daily varied from 0.05 gram to 0.3 gram, each group in the litter being kept on the same doses.

TABLE 1

Average weight of rats in litters A to K (8 litters, 56 rats, 30 males and 26 females)

Weight in grams

WEEK	30 MALES				36 FEMALES			
	Anterior lobe (9)	Posterior lobe (6)	Whole pituitary (7)	Brain (8)	Anterior lobe (6)	Posterior lobe (7)	Whole pituitary (5)	Brain (8)
1	41	41	37	40	41	37	36	41
2	53	51	47	50	55	47	46	51
3	69	67	61	65	69	62	60	66
4	77	74	68	74	79	73	73	71
5	92	90	86	94	93	83	75	91
6	108	106	100	111	108	93	85	100
7	122	117	114	120	123	104	98	114
8	131	128	123	132	129	114	103	121
9	145	143	135	145	125	115	112	126
10	158	162	151	156	125	126	128	128
11	154	157	149	148	118	123		125
12	171	168	167	158	129	131		129
13	173	175	173	167	132	127		126
14	190	189	188	175	147	130		129
15	189	185	200	182	146	130		131
16	197	187	211	193	171	137		139

The material was made up into a bread pill and fed to each rat by hand before giving the rats their general daily ration. In this way we were assured that the material fed was actually ingested. The rats never refused the material.

The stock diet consisted of whole grain, bread, milk and lettuce ad libitum.

Results. In the first series groups (one male and one female, so far as possible) were fed on anterior lobe, posterior lobe, whole pituitary and brain. The summary of weights and growth of the rats in this

series is given in table 1. This table shows no definite influence of pituitary feeding on the body growth. There was no demonstrable difference in the general activity of the rats in the different groups. Sometimes one or two individuals outgrew the others of the same litter, but this happened just as frequently in the brain-fed groups as in the pituitary-fed groups. When the stock diet is adequate in quantity and quality, individual differences (probably hereditary) in growth

TABLE 2

Average growth and gonad development of 8 litters of rats (25 males and 24 females) to the stock diet of which was added anterior pituitary, whole pituitary, or brain substance. The feeding was started with each litter at 4 weeks of age, and continued for 4 weeks, when the animals were killed for examination of the gonads, vas and uterus. During the feeding period the rats in this series were weighed every other day.

Weight in grams

DAYS	25 MALES			34 FEMALES		
	Anterior lobe (10)	Whole gland (8)	Brain (6)	Anterior lobe (8)	Whole gland (6)	Brain (10)
0	34	31	34	33	32	33
2	39	35	39	38	35	37
4	42	38	44	39	39	42
6	46	42	49	43	44	48
8	48	44	52	45	45	58
10	52	48	56	51	51	54
12	56	52	61	55	55	59
14	59	58	66	58	61	62
16	63	61	70	63	65	65
18	69	68	73	63	66	66
20	75	74	79	69	70	70
22	78	76	82	70	72	74
24	85	83	88	75	74	79
26	89	75	85	80	75	79
28	90	85	88	80	77	84

energy outweigh any slight influence that may be exerted by the ingestion of desiccated and de-greased pituitary gland.

In the second series posterior lobe feeding was eliminated, leaving the anterior lobe, whole pituitary groups, and the brain controls. All the rats of this series were 2 months old when killed after a feeding period of 4 weeks. The feeding period was made short in order that the gonads might be examined before sexual maturity. This is obviously necessary if one is to secure definite evidence of stimulation of gonad

TABLE 3

Final body weight and gross weight of ovary-uterus and testes-vas, in 8 litters of rats fed for 4 weeks as per table 2

All weights in grams

LITTER	MATERIAL FED	MALES Body weight	MALES Testes-vas	FEMALES Body weight	FEMALES Ovaries-uterus
L	Anterior lobe	78	1.520	81	0.320
	Whole gland	81	1.265	83	0.220
	Brain	77	1.515	85	0.100
	Brain			80	0.100
M	Anterior lobe	90	1.495		
	Whole gland	90	1.785		
	Whole gland	84	1.540		
	Brain	75	1.360	93	0.305
N	Anterior lobe	90	1.560	72	0.155
	Anterior lobe			68	0.130
	Whole gland	76	1.370	73	0.140
	Whole gland			82	0.245
	Brain	103	1.625	74	0.140
	Brain			76	0.125
O	Anterior lobe	106	2.075	97	0.155
	Whole gland	99	1.655		
	Whole gland	111	1.910		
	Brain	125	2.150	91	0.290
Q	Anterior lobe	67	0.880	60	0.060
	Whole gland	80	0.965	57	0.080
	Brain	81	1.290	67	0.060
R	Anterior lobe	59	1.020	43	0.055
	Anterior lobe	54	1.160		
	Whole gland	62	0.640	73	0.115
	Brain	67	1.020	73	0.115
	Brain			52	0.080
S	Anterior lobe			88	0.235
	Anterior lobe			74	0.200
	Whole gland			83	0.445
	Whole gland			80	0.120
	Brain			79	0.110
	Brain			94	0.225
T	Anterior lobe	61	0.815		
	Anterior lobe	59	0.605		
	Whole gland	64	0.945		
	Whole gland	61	0.710		
	Brain	76	1.520	66	0.155

activity by the pituitary feeding. The results of this series are presented in summaries in tables 2 and 3. Table 2 reveals no influence on body growth from the pituitary feeding. Table 3 records the final weight of each rat, and the combined weight of testes-vas and ovary-uterus. An examination of this table shows marked individual variations in testes-vas and ovary-uterus development, but no distinct stimulation of gonad growth by the pituitary feedings. This conclusion was also substantiated by the histological examination of the ovaries and testes. As in the case of the first series, in regard to the body growths, the individual growth potentialities of the gonads outweigh and obscure any slight stimulation from the pituitary feeding, when the diet is otherwise adequate.

SUMMARY

When desiccated pituitary gland substance (whole gland, anterior lobe, posterior lobe) is fed in quantities from 0.05 gram to 0.30 gram per day for 4 to 16 weeks to white rats 1 month old, and on an adequate dietary, the pituitary feeding has no influence on body growth or gonad development.

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THE EFFECT OF QUININE ON THE IODINE CONTENT OF THE THYROID GLAND

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It has been well established that the morphology and iodine content of the thyroid gland undergoes seasonal variations in certain animals. On the basis of these observations Mills (1) determined the effect of high and low temperature, stimulants and depressants on the morphology of the thyroid gland. Of particular interest was the observation that quinine produced changes in the gland suggesting diminution of activity, namely, a flattening of the epithelium and increase in amount and apparent density of the thyroid colloid. The logical conclusion from this quinine effect is that it results directly from depression of protein metabolism, and by logic this would indicate that one form of thyroid stimulant is dependent upon protein metabolism.

Mills' criterion of thyroid change in activity consisted in morphological differentiation. Iodine analyses were not made. It is true however that iodine concentration and morphology do not always run parallel and furthermore both iodine concentration and morphological changes may fail to represent a true record of passed activity of the gland. Be that as it may, they constitute the best evidences at present available. Changes in iodine content or in morphology mean some fundamental change in activity while on the other hand certain changes in activity may well occur without detectable chemical or morphological alteration.

Lack of iodine determination in Mills' study together with lack of consideration of inanition and its effects following quinine exhibition leave open certain phases of the question to which this paper is devoted.

For purposes of expediency dogs were used to determine the changes in iodine content of the thyroid glands resulting from quinine exhibition. Normal dogs were chosen as much as was possible in order to avoid the complications of goiter, though it is not at all probable that hyperplastic glands differ in response to quinine from "normal" glands except in a quantitative way.

The dogs were kept for several days under ordinary laboratory conditions with the usual diet for stock animals. Care was taken to avoid iodine contamination but cognizance is taken of the impossibility of maintaining absolute iodine quarantine in a laboratory where iodine has been used for many years in routine and emergency work on animals. The most that could be hoped for was that control animals would give a reliable index of unavoidable contamination and should the test animals give changes significantly different from the control series then some safe conclusions may be drawn.

One lobe of the thyroid gland was removed asceptically under ether anesthesia from control and experimental animals. Controls were kept during the same time and in the same quarters as the experimental animals. Food and water were given to each set from the same supply.

The experimental animals were given daily 0.2 to 0.3 gram per kilo, quinine sulphate in capsules. The animals were carefully watched and in case vomiting occurred the quinine was given in divided doses several times a day. After 6 to 8 days the animals were anesthetized, the remaining gland removed, and the animals then killed. Aseptic precautions seemed superfluous at this point.

From both control and test lobe small sections were taken for morphological examination, while the balance of each lobe was dried in the drying oven and vacuum desiccator to constant weight and analyzed for iodine by the Kendall technique (2).

From the table it may be seen that the iodine content and concentration was increased slightly in the control unquininized series while in the quinine series the iodine increased remarkably and without exception. The increase of iodine in the quinine series was so great that there was no room for doubt of the effect of quinine on iodine accumulation in the thyroid.

Morphological changes in general paralleled the iodine changes, though by no means so consistently or significantly. These findings bear out the common view that morphological state is not a true index of iodine content although in general it is fairly indicative.

Since the animals under the influence of quinine were frequently depressed and ate but little the question of inanition or starvation required additional control. Consequently the above experiments were repeated with the difference of withholding food from both groups, namely, fasting controls and fasting plus quinine. Water was freely supplied.

In this series it was found that in the controls there was a diminution in amount of colloid material, a heightening of the epithelial cells

increased vacuole formation and on analysis for iodine it was found to be decreased. On the other hand, the thyroid of the quininized fasted animal was richer in colloid, cells flatter, and iodine content increased. The increase of iodine in the quininized fasting animal was relatively rather less than obtained in the quininized animals which received food.

The effect of high temperature on iodine changes was next given consideration. For this, animals were kept in an appropriate box in which the temperature was kept at approximately 35°C. throughout the experimental period. Water but no food was supplied. Again control and quininized animals were prepared as usual and later compared as to morphology and iodine content of the thyroid glands.

TABLE 1

Condensed table of results of study of the changes in iodine percentage in the thyroid gland of dogs under the influence of quinine, starvation and high temperature

SERIES	NUMBER OF ANIMALS	LOBE	PER CENT IODINE (AVERAGE)	PER CENT CHANGE	NOTES
I	10	Control	0.0973		Control to series II
		Exper.	0.1079	+10.9	
II	10	Control	0.0702		
		Exper.	0.1369	+95.0	Quinine treated
III	3	Control	0.034		
		Exper.	0.22	−35.2	Fasting. Control to series IV.
IV	5	Control	0.0376		
		Exper.	0.0600	+59.5	Fasting, with quinine
V	2	Control	0.058		
		Exper.	0.133		Fasting at high temperature control to series VI
VI	2	Control	0.177		
		Exper.	0.167		Fasting, quinine, high temp.

In this series, though small in number and possibly equivocal as to interpretation, it is found that whereas in the control the total iodine was increased, in the quininized animal the thyroid iodine was not significantly changed.

Having clearly demonstrated that changes in iodine content of the thyroid gland by quinine medication do occur, the question arises as to whether the changed iodine balance in the body is inherent in direct

thyroid changes or in changes in the body as a whole, for example, changes in metabolism which act as the immediate agency in altering the iodine distribution in the body. If quinine has a direct primary action on the thyroid gland, acute quininization might well be expected to alter the affinity of the thyroid for iodine injected directly into the circulation. (Marine technique (3).)

For this purpose light ether anesthesia was employed. For purposes of economy of space, tables are not herein recorded but instead our own deductions are presented.

Intravenous injections of quinine alone appeared to have no immediate effect on the iodine content or percentage. In a series of seventeen experiments, a previous injection of quinine had no effect whatsoever on the capacity of the thyroid gland to absorb iodine from intravenously injected potassium iodide solutions.

Since acidosis is a natural consequence of starvation which in turn occurs in some measure in the series given above, it is quite desirable to obtain some evidence on this point as a possible complicating factor.

Rabbits were used in this series using morphological appearance as the criterion for thyroid change. A small specimen of thyroid tissue was removed aseptically during ether anesthesia. After a few days the animals were subjected to appropriate conditions deemed likely to indicate thyroid changes subsequent to starvation and acidosis. For a measure of acidosis the alkaline reserve capacity of whole blood was used.

In group I, all animals received food and water, while the test animals were given daily gastric administrations of 100 cc. of 1 per cent HCl. In group II, the animals were supplied water but no food. The test animals were given 100 cc. 1 per cent HCl by stomach daily.

The results of this series of experiments permitted the conclusion that starvation alone or acid administration with or without food caused colloid diminution, and increase in cell height with evidences of degenerative cell changes occurring in some. Fasting animals receiving acid were much more severely affected than animals receiving food, particularly in respect to degenerative changes. However, morphological changes in the thyroid occurred in starvation even when the alkaline reserve capacity of whole blood did not change. From this evidence the conclusion is warranted that in the rabbit, at any rate, starvation affects the thyroid gland by some means other than acidosis as detected by reserve capacity measurements.

Discussion. The observations of Mills on the influence of quinine and high external temperature on thyroid morphology is confirmed and

extended by the additional finding of an actual increase of iodine. Starvation studies of Jackson (4), Vincent and Hallenberg (5) were likewise confirmed in that signs of degenerative changes may occur. Furthermore iodine is found to have been diminished in the thyroid of fasting animals. Unilateral thyroidectomy appears to lead to an increase in iodine in the remaining lobe.

These evidences lead one to consider the existence of a type of iodine equilibrium in the body, the actual distribution being dependent in some way upon metabolism. Since quinine, fasting and high temperature surroundings are potent agents in causing a change in distribution of iodine it would appear that endogenous protein metabolism may well be the source of the immediate agency of thyroid control.

SUMMARY

1. Both quinine and high external temperature cause an increase in the iodine content and concentration in the thyroid gland while a decrease is produced by starvation.

2. These facts are correlated in the view that endogenous protein metabolism is fundamentally responsible for the distribution of iodine in the body.

The writer wishes to express his thanks to Dr. A. L. Tatum for the suggestion of this problem and for helpful criticisms during the investigation.

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LIPASE PRODUCTION BY THE LIVER

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Some previous unpublished work of the author suggested either new production of lipase by the liver or augmentation of activity of the lipase already present there. Unfortunately, the experimental conditions involved variables too numerous to permit of conclusions bearing directly upon this point.

A thorough review of the literature reveals the fact that the lipolytic content of various organs has been determined, but no attempt has been made to determine the source of this lipase.

The present research was executed, therefore, to ascertain whether or not the liver is a possible source of lipase. With the present meager knowledge of enzymes in general and lipase in particular, it is impossible to know whether a quantitative increase has occurred or that the activity of the enzymes has merely been enhanced when increased enzymic activity is manifested. Subsequent reference to lipase production by the liver will denote that there has occurred an increased lipolytic activity by that organ.

Experimental method: *Selection and preparation of animals.* Small dogs were used throughout this investigation for two reasons; first, there was found to be, as far as this experiment is concerned, a greater proportionate amount of available blood in relation to the liver mass than in medium size animals, and second, it was found to be much easier to decapitate small dogs than larger ones.

The age of the animals was given no consideration except that no suckling puppies were used.

Although no particular attention was given to the previous nutritional condition of any of these animals, they were all given their last food about twenty hours before the experiment was begun.

Choice of method. To determine any change in the lipolytic activity of the liver, per se, only two general methods are open to choice; *1*,

attempt to vary the lipolytic activity of the liver, in situ, and determine such variations in liver samples and in the blood flowing therefrom; or 2, isolate the liver and perfuse it with defibrinated blood containing substances capable of exciting variations in the lipolytic activity of the liver. In the first instance, the possibilities of lipolytic activity being altered in tissues other than the liver and affecting this factor in both blood and liver are so great as to preclude the practicability of this method. While the gross disadvantages of any perfusion method are universally recognized, there seemed to be no other method open to choice. It was hoped at the outset that any variations obtained would be of great enough magnitude to negative any natural errors attributable to the perfusion method.

Experimental procedures common to all series. The dogs, having been fed about twenty hours previously, were decapitated. The severed neck was placed over a receptacle and the blood obtained from the body was defibrinated by an assistant. The liver, which appeared to be completely exsanguinated, was mounted in the perfusion apparatus and perfused through the portal vein with the defibrinated blood which was properly diluted with Ringer's fluid to obtain adequate volume. The average time which elapsed between the decapitation and the beginning of the perfusion was twelve minutes.

Experimental facts concerning agents capable of producing variations in the lipolytic activity of the liver do not exist. Therefore it was necessary to employ agents of questionable ability in this respect. Accordingly, this investigation was divided into four series as outlined below. Each series comprises the livers treated with agents considered most likely to cause variations in the hepatic lipase content.

Technique of perfusion. Immediately after excision of the liver it was mounted in the warm chamber of the perfusion apparatus, the cannula being tied into the portal vein.

The defibrinated blood mixture was transferred to the proper cylinder where room air bubbling through it kept it properly oxygenated.

This blood mixture was forced by air pressure of 45 to 50 mm. Hg through a glass tube surrounded by water at a constant temperature of 40°C. The liver was also kept constantly at this temperature.

The high humidity prevailing within the warm chamber was evidenced by condensation of moisture upon the glass door of the chamber.

The passage of air into the liver was precluded by means of a small by-pass, just above the liver, through which air was allowed to escape.

Foam arising from the bubbling of air through the blood and passing into the liver was obviated by forcing blood from the bottom of the cylinder and not using that portion of the blood laden with foam.

Procedure in series 1. Series 1 comprises control experiments to determine the variations, if there be any, and the limits of such variations in the lipolytic activity of the liver when this organ is perfused with normal, diluted, defibrinated blood, continuously, over a period of two hours. Samples of both blood and liver were taken at varying intervals during this time for quantitative determinations of their lipolytic activity.

Procedure in series 2. Evidence supporting the contention that some intact viscera are excited to greater activity by the effects of asphyxia, it was decided to use this agent as a possible factor for altering the fat-hydrolyzing power of the liver. To accomplish this end, the liver was perfused for a short time with the normal blood mixture. The perfusion was then stopped for a period of approximately one hour after which it was again perfused with the normal blood mixture. The samples, which were taken at various intervals in the course of the experiment, were tested for their lipolytic activity.

Procedure in series 3. The livers in this series were perfused for a relatively short interval. Then a small amount of secretin,[1] in Ringer's fluid, was added to one-half of the normal blood mixture. After perfusing with this blood-secretin mixture for a short time, perfusion with the normal fluid was resumed and continued until the termination of the experiment.

Procedure in series 4. After the liver had been perfused for a brief period with the normal blood mixture, the blood mixture was divided into two equal parts. Pilocarpine (2 or 3 mgms.) was added to one part and this was perfused for a short period. After this perfusion was discontinued, the liver was again perfused with the normal blood mixture.

Method of lipase determination in liver. The method used for the quantitative determination of lipase is essentially the same as that used by Kastle and Loevenhart (1).

The results given in this paper are derived from aqueous extracts or "brei" of the fresh liver substance.

The extract was prepared as follows: 5 grams (weighed to balance on a Sartorius balance) of fresh liver substance were ground with 10 grams

[1] The secretin was prepared by boiling in 200 cc. of 0.4 per cent HCl the ground mucosa of the duodenum and the upper ileum of a dog, neutralizing with KOH and filtering. The neutral filtrate was added to the blood mixture and passed through the liver.

of chemically clean sand and diluted with 100 cc. of distilled water for extraction to occur. Extraction proceeded for 24 hours. It was observed that the supernatant aqueous extract of the first liver samples (the unperfused liver sample) was always turbid whereas that of the perfused samples was always clear. One cubic centimeter aliquots of the supernatant fluid were incubated for one hour in ground glass stoppered weighing tubes with 10 cc. of distilled water, 3 drops of toluol, and 2 drops of neutral azolitmin. The tubes were removed from the incubator and neutralized with KOH. Five-tenths cubic centimeter of neutral, absolute ethyl butyrate was then added and the tubes incubated at 40°C. At the expiration of 24 hours the tubes were removed and their contents titrated against N/50 NaOH to determine the amount of butyric acid liberated by the hydrolysis of the ethyl butyrate.

Checks were run on the amount of ethyl butyrate hydrolyzed, the amount of acid liberated spontaneously by the liver and the hydrolysis of the ethyl butyrate by water. These factors are all considered and calculated for in the preparation of graphs showing the results. The figures given, therefore, represent the lipolytic activity of 1 cc. of the aqueous liver extract and 1 cc. of the supernatent fluid from the centrifugalized blood samples in terms of N/50 NaOH.

Method of lipase determination in blood. After completion of the perfusion, all of the blood samples taken during this procedure were centrifugalized at one time. One cubic centimeter samples of the supernatant fluid, which were composed of blood serum, Ringer's fluid, etc., were then treated in the same way as the samples of the aqueous extract of the liver for quantitative determination of lipolytic activity.

EXPERIMENTAL RESULTS: *Series 1.* This series comprises the control experiments in which the livers were perfused continuously for a period of two hours with defibrinated blood diluted (two parts of blood to one of Ringer's fluid) with Ringer's fluid.

The average of results obtained in this series is embodied in figure 1.

As shown in figure 1, the lipolytic content of the blood is low while that of the liver is high when samples are taken before perfusion is commenced.

During the initial short perfusion period the hepatic lipase falls markedly. Although the blood lipase rises at this time, its increase hardly seems commensurate with the hepatic decrease.

Following the initial perfusion period the liver value rises very slightly for a short time after which it continues to fall gradually to the con-

clusion of the experiment. The blood lipase in all but one animal increased slightly until the perfusion was interrupted.

Series 2. In this series the livers were perfused initially with the normal perfusion fluid. Perfusion was then suspended for one hour, later to be resumed. This asphyxial period or interval during which perfusion was suspended was chosen as a possible agent to vary the lipolytic activity of the liver.

Figure 2 shows the average of the results obtained in this series.

As in the previous series, the liver showed a marked initial fall in lipase and the blood a small rise in lipase while being perfused. Asphyxiation for one hour resulted in only a slight rise in the blood lipase and if any appreciable change in the hepatic lipase a mere tendency toward a decreased activity.

These results are practically the same as those obtained in the control experiments, series 1.

Series 3. In this series a dilute solution of secretin was added to the normal perfusion mixture. Because this body is known to act as an excitant to the pancreas, it was decided to try it for a similar action upon the lipolytic activity of the liver.

The average of results obtained from this series of animals is shown graphically in figure 3.

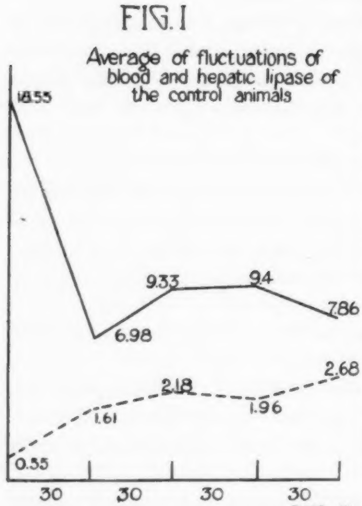
Throughout this series there was the same sharp fall in the lipase content of the liver, as in the control experiments, after the initial perfusion with the normal blood mixture. Accompanying this decrease there was a well-marked but not proportionate increase in the lipolytic activity of the blood.

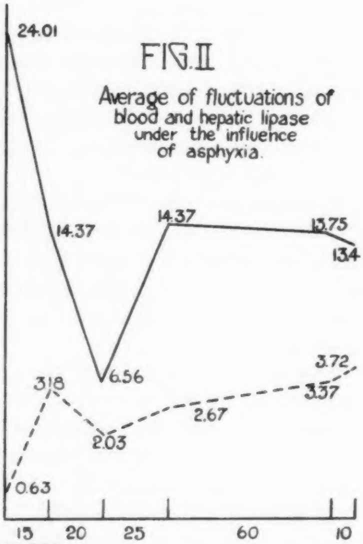
After the initial normal perfusion of the liver this process was interrupted for about an hour, in most instances, while secretin was being prepared from the intestine of the same dog. The results of this asphyxial period were identical with those of series 2.

A measured amount of secretin was added to the blood mixture for perfusion. The lipase value of the blood usually fell slightly, due probably to the effect of dilution.

Perfusion of the liver with this blood-secretin mixture resulted in a marked rise in blood and hepatic lipase, simultaneously, or in the liver first and later in the blood in all but one instance. This exception is easily explained by the fact that the secretin used had been kept in an open vessel at room temperature for five days.

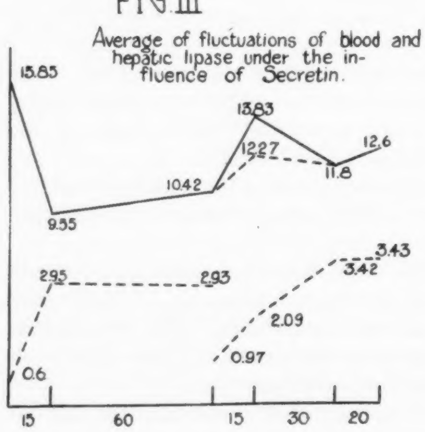
The above increase usually occurred within fifteen minutes and did not persist at the same high level in the liver when perfusion with secretin was continued for as long as one-half hour.

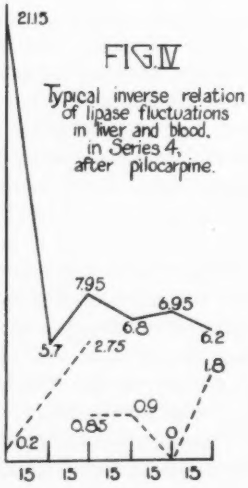




TIME IN MINUTES.

—LIVER } ---BLOOD } Lipase in terms of n/50 NaOH per cc of extract.





While in the typical experiments secretin produced a marked increase in the lipase of the liver uniformly there was one instance in which this factor was increased almost 40 per cent.

The liver was then perfused with the normal blood mixture. This was followed, in the majority of instances, by a slight rise in the hepatic and a greater rise in the blood lipase.

Series 4. Pilocarpine (1 or 2 mgm.) was added to the normal perfusate and passed through the liver in an attempt to vary the fat-splitting activity of this organ.

The results of one experiment, typical of this series, are shown graphically in figure 4.

Perfusion with pilocarpine was followed by a rise in hepatic lipase in only one animal. In this case the rise was small enough, probably, to fall within the limits of experimental error. In the typical experiments there occurred either no appreciable change or a very slight decrease.

The mere addition of pilocarpine to the blood, with one exception, depressed its lipolytic activity appreciably. The tendency of this blood to alter its activity when passed through the liver was small, but such variations were present and about equally divided between an increase and a decrease.

There was a definite tendency for the liver to decrease and the blood to increase in lipolytic activity upon resumption of perfusion with normal blood mixture subsequent to the perfusion with pilocarpine.

The interesting feature of this series is shown in figure 4, in which increases or decreases in the liver lipase were, practically speaking, always accompanied by an inverse reaction in the blood.

SUMMARY AND CONCLUSIONS

1. The isolated liver is unquestionably capable of increasing and decreasing its lipolytic activity when perfused with *a*, a defibrinated blood-Ringer's mixture; *b*, blood-secretin mixture and *c*, blood-pilocarpine mixture.

2. Lipolytic activity of the liver is not altered immediately by asphyxia, but changes are induced which are favorable to increased activity during subsequent perfusion.

3. The liver produces lipase when perfused with a blood-Ringer's mixture containing fresh secretin, the maximal increase mounting to nearly 40 per cent in one instance.

4. Although hepatic lipase does not seem to be increased by the action of pilocarpine, interchange of lipase between liver and blood is apparently facilitated under its influence.

5. The lipase content of the unperfused liver is very much greater than that of the perfused liver.

6. The marked decrease in hepatic lipase following the inital perfusion with normal blood mixture does not seem to be accounted for by the slight increase in blood lipase.

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THE INFLUENCE OF THYROID EXTRACTS AND THYROXIN ON THE RATE OF MULTIPLICATION OF PARAMECIA

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The influence of thyroid substances upon growing organisms was first shown by Gudernatsch (1) in his experiments on tadpoles. Those experiments gave us an insight into the action of thyroid substances on organ development. Experiments with thyroid extracts on protozoa seemed to offer a means for understanding the mechanism of the action of the thyroid substance upon the cells themselves. The first attempt in this line was made by Nowikoff (2) who found that thyroid extracts accelerated the rate of multiplication of paramecia. Since his controls were cultivated in distilled water or 5 per cent egg albumen, they were incomparable with those animals cultivated in thyroid extracts. Furthermore, the number of his experiments was insufficient to draw any definite conclusions. While our work was in progress we found a recent publication of Abderhalden and Schiffman (3) dealing with the same problem. These authors investigated the action of "optons" on the division rate of paramecium caudatum, using the following technic: their culture medium was hay infusion to which the optons were added in a concentration of 1:1000. The pure infusion served as a control. Their cultures were kept for 8 to 9 days without transplantation and the number of paramecia present reported daily. This technic finally necessitated the estimation of as many as 500 paramecia in one culture. The authors summarize their data drawing a logarithmic curve from each of their six experiments. Therefore, every curve corresponds to the multiplication rate of only one culture. These curves indicate that the rate of multiplication of the paramecia is markedly increased under the influence of thyroid opton.

Abderhalden and Schiffman's technic, however, differs in two very important points from that developed by Woodruff (4), Jennings (5), Robertson (6) and other investigators who transplanted every 24 hours and averaged a number of sister cultures to obtain consistent data.

Since the review of the available literature does not afford very conclusive data on the influence of thyroid extracts on protozoa, this work was undertaken to further investigate this question with the additional object of establishing the relationship between the action of thyroxin (Kendall) and the thyroid extracts.

EXPERIMENTAL. In the following experiments the technic of Robertson and Woodruff was followed, which minimizes the errors introduced by the variability of the test animals. A "wild" paramecium putrinum was isolated from a hay-infusion and only the descendants of this, forming a pure strain, were used. Copulation did not occur during the experimental period. As culture medium a hay infusion was used. This was prepared by adding 100 cc. of tap water to 5 grams of dry hay bringing it to a boil and then keeping it for 20 minutes on a boiling water bath. To 10 cc. of the filtered infusion was added 0.4 cc. n/10 Na_2CO_3. This medium was used fresh or kept from 2 to 8 days in the ice box, since it was found that no appreciable bacterial growth took place at that temperature. The paramecia were cultivated on concave slides and kept in a moist chamber. All glassware employed was sterilized. One individual only was transplanted every 24 hours to each new culture and great care taken to obtain the same size of drop in all cases (about 0.08 cc.). For the daily transplantation the directions of Robertson were followed which have the advantage of carrying over only a very small amount of fluid and bacteria from the old to the new culture.

Thyroid powder (Parke, Davis & Co.) was added to the infusion in concentrations of 1:100, 1:200, 1:300. After shaking for 30 minutes the suspension was centrifuged or allowed to settle and the supernatant fluid decanted. In a few cases filtration was used. Hay infusion without thyroid and treated in the same manner served as control. Thyroxin, which unfortunately is very insoluble in fluids of a pH compatible with life, was applied in two ways: a small crystal of about 0.1 to 0.2 mgm. was added to 2 cc. of hay infusion and heated for five minutes with the control hay infusion in a boiling water bath: in the other case a weighed amount of thyroxin was dissolved by heat in the Na_2CO_3 solution used for neutralization. This solution was added to the unneutralized hay infusion, the final concentration of thyroxin being 1:100,000.

Two series of data were collected: in one the number of paramecia was counted after 24 hours and the value of 16 to 20 sister cultures, half of which were controls, recorded. The average value of the con-

trol was fixed at 100, thus the relation of thyroid (thyroxin) to control evaluated. In the other series the conclusions were drawn, in accordance with the technic of Woodruff, from the number of divisions.

For the first series it was found essential to transplant paramecia of the same age, in order to obtain consistent values in the sister cultures. Animals just formed by division were chosen for transplantation.

The paramecium putrinum used divided itself 4 to 5 times at 24°C., the temperature at which most of the experiments were carried on. The paramecia were counted with a 32 mm. objective and the following method found very useful for controlling the count. The method was based on the fact that a paramecium just formed by division is about one-half the size of a mature animal. Therefore, there must be a definite relation in the number of large and small animals present; i.e., if the count was 35 the ratio of large to small animals must have been 29 to 6. By this method one could estimate accurately as many as 64 paramecia per culture. Paramecia in the division stage were counted as 1½.

Experiments with thyroid extracts. *1.* With thyroid extract 1:100 five experiments were made in which ten paramecia were kept under the influence of thyroid extract, ten as control. As illustration a full experiment is described:

8/21-22 Culture no. 33, 20 sister cultures. Paramecia after 24 hours:

Control:	16	33	16	29	16	17	16	16	20	28½
Thyroid 1:100:	31	32	58	60	32	64	35	34	61	32

In those five experiments the numbers of paramecia averaged from 20 sister cultures for each experiment gave the following figures:

For the control:	21.9	29.7	20.7	36.5	26.6
For thyroid 1:100:	42.5	32.4	43.9	39.2	48.0

The average ratio control: thyroid = 100:160.

2. Eight experiments were made with thyroid extract 1:200 the results being as follows:

For the control:	15.1	18.6	12.6	12.4	10.0	10.9	22.9	43.1
For thyroid 1:200:	22.5	27.0	20.0	20.8	16.0	13.2	32.6	49.5

The average ratio control: thyroid = 100:144.

3. Three experiments were carried out with thyroid extract 1:300. The average ratio control thyroid = 100:141.

Experiments with thyroxin. Thirteen experiments were carried out for which the average number of paramecia was:

For the control:

12.0	10.9	14.2	35.8	14.6	8.1	29.0	10.5	30.6	17.1	9.2	29.9	45.1

For thyroxin:

14.4	13.2	18.2	36.0	17.6	10.8	24.0	12.3	31.7	22.1	10.5	15.9	55.8

The average ratio control thyroxin = 100:112.

The data recorded show that the multiplication rate of paramecia kept in thyroid extract is always greater than in the corresponding control. It will be further observed that the number of multiplications often varies considerably from day to day. These variations were the most noticeable during periods in which great differences of room temperature occurred. However, no attempt was made to keep the animals at a constant temperature since experiments showed that temperature changes had no appreciable effect on the action of the thyroid extract. Previous investigators have shown that paramecia even under the same experimental conditions have a variable susceptibility to stimuli at different periods of their life cycle. This probably explains the variations in the intensity of thyroid action found in the experiments recorded.

The effect of thyroxin on the multiplication rate of paramecia is not as strong nor as consistent as that of thyroid extract, even though the concentration of thyroxin used (1:100,000) is, according to Kendall, equivalent to 1:100 thyroid extract.

In the second series of experiments the number of divisions over a period of 4 to 5 days was observed. Each series consisted of ten or fifteen sister cultures, five of which were controls. The paramecia were transplanted every 24 hours.

The data are summarized as follows:

Number of divisions in 24 hours averaged from 5 sister cultures in 5 days.

For the control:	4.53	4.87	3.53	5.00	5.04
For thyroxin 1:100,000:	4.72	5.00	—	4.95	5.22
For thyroid 1:200:	—	—	3.91	5.40	5.70

The average ratio control: thyroxin: thyroid = 100:102:112.

It will be seen that this technic also confirms our previous results. Here again thyroxin less intensely influences the multiplication rate of the paramecia.

SUMMARY

1. Faintly alkaline thyroid extract in hay infusion strongly accelerates the multiplication rate of paramecium putrinum.

2. Equivalent concentrations of a solution of thyroxin in hay infusion only very slightly accelerate the rate of multiplication.

3. These experiments indicate that thyroid extract contains another active substance besides thyroxin, which accelerates the division rate of paramecia. Therefore this furnishes no evidence that the acceleration is due to a specific substance.

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THE EFFECT OF TENSION ON THE ACTION CURRENT OF SKELETAL MUSCLE

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In a research now in progress in this laboratory, of which a preliminary report has already appeared (1), the size of the action currents of a muscle is used as a criterion of the relative number of fibers simultaneously excited by motor-nerve stimulation under various conditions. The purpose of this was to deduce by elimination the number of fibers at the moment refractory or innervated by nerve fibers in a refractory state, under various conditions of reflex activity. It was suggested by Doctor Drinker that this issue might be confused by possible changes in the size of action currents due to mechanical tension on the muscle fibers resulting from the reflex contraction. We therefore undertook to find out whether the amplitude of the action current in response to a single maximal break shock might be varied by the amount of initial tension passively imposed upon the muscle.

It is a familiar classroom experiment to observe the increase in mechanical work done by a single twitch in consequence of increasing tension. Starling (2) stated that the energy of contraction of heart muscle increased as the volume of the heart increased. The augmentation of energy was thought by him and others to be caused by an increase in the length of the fiber and not by any increase of initial tension. Blix (3) held this same view for striated muscle. Wiggers (4) maintained that in the case of heart the tension is the predominant factor.

These observations refer to mechanical response. Our problem deals with the electric response. Du Bois-Reymond (5), Meissner and Cohn (6), Lamansky (7), Schenck (8), Bernstein (9) and Buchanan (10) have all made observations of action currents produced by tetanizing stimuli with muscles under varying degrees of tension. But their results have only an indirect bearing on the point at issue, for they are complicated by the question of the stage of recovery of the individual

muscle fiber from its previous response and by other possible factors not involved when single stimuli are used. The question in hand, viz., the action current in response to a single instantaneous stimulus, may have little or nothing to do with the tension actively developed by the contraction of the muscle, for it is well known that this action current has passed its maximum before the mechanical response has appreciably altered the tension.

Some observations, however, are on record dealing with single stimuli, and therefore bearing more directly on our problem. Lamansky (7), working on the gastrocnemius muscle of the frog, found an increase in the size of diphasic action currents in response to single shocks, on increasing the initial tension up to 180 grams, at which a maximum was reached. Further increase in tension caused a decrease in the size of action current. Rivière (11), using the gastrocnemius muscle of the frog and a capillary electrometer, also found an increase in the diphasic action current with increase in tension. Burdon Sanderson (12) recorded the action currents, both diphasic and monophasic, of the curarized sartorius of the frog with a capillary electrometer and found no distinct change in the size of response up to 15 grams tension. Bernstein (9), who used the adductor muscles of the frog, muscles which, like the sartorius, have parallel fibers, reported an increase in the monophasic action current with increase in tension; and later Bernstein and Tschermak (13) found that the action current of these muscles passed through a maximum as the initial tension was increased. Samojloff (14), also with the capillary electrometer, observed an increase in the diphasic action current of the frog's gastrocnemius muscle when tension was applied to it; this action current reached a maximum which was sustained as tension was increased up to 200 grams, beyond which no further increase in tension was tried. He also found (15) that when, during an isotonic contraction, a second stimulus is applied to a frog's gastrocnemius muscle at a short interval after the first, there is, following the relative refractory phase, a period when the second action current is smaller than the first, showing a minimum at the time of greatest shortening of the muscle. This effect was absent if the contraction was made isometric. This suggests that the development of tension in the isometric contraction tends to increase the size of action current.

Schenck (8) observed that if while a muscle (either the gastrocnemius or sartorius) was tetanized with slow frequency, the tension was made alternately great and small, the monophasic action currents increased

in magnitude during tension. He also found that the resting or demarcation current was decreased during tension. Einthoven (16), (17) reported electrical changes correlated with change of shape in resting muscle, and stated that the demarcation current decreases with tension in the gastrocnemius of the frog, but increases with tension in the sartorius of the frog, and in the gastrocnemius of the cat. In the case of the frog's gastrocnemius De Meyer (18) has made extensive studies of these electrical changes which he designates deformation currents. In general he finds that when tension is applied to a muscle the part of the fibers which undergoes the greatest stretching acquires a relatively positive potential. If we may apply to the muscle the conceptions of the membrane theory of functional response (19), this change may be looked on as an increase in the demarcation current. For, according to this conception, the demarcation current depends on the breaking down of the polarized membrane of the fiber at the injured point, resulting in a local short-circuit between the positive outside and the negative inside of the fiber. If the stretched part of the fiber becomes positive with respect to an unstretched adjacent part this suggests an increase in the difference of potential between the inside and the outside in the stretched portion. If the propagated disturbance involves the temporary breaking down of the polarized state of the membrane, then the result of increasing the amount of polarization under tension would be to increase the possible magnitude of the action current.

These considerations, however, involve assumptions too speculative to be given much weight. Moreover, it should be noted that the above interpretation of the potential change observed by De Meyer in the stretched gastrocnemius does not harmonize with the decrease of demarcation current in this muscle observed by Einthoven, who suggests another explanation for the difference between the sartorius and gastrocnemius muscles (17, p. 138). The conditions on which both demarcation current and action current depend, and the precise relation of one to the other are not well enough known to enable us to predict with any confidence the effect which the deformation current would have on the size of the action current induced in a stretched muscle.

Certain other work which has merely an indirect bearing on the problem under discussion should perhaps be mentioned here. Eddy and Downs (20) found that prolonged stretching of a frog gastrocnemius muscle hastens the onset of fatigue due to subsequent stimulation.

They found also that the stretched muscle produces CO_2 at a faster rate than the unstretched muscle under similar conditions.

Hieronymus (21) measured the velocity of the propagated disturbance sweeping down the muscle fibers of a curarized frog sartorius and found that when the muscle was put under tension and stretched, the time taken by the disturbance to traverse the length of the muscle was increased; but his results did not determine with certainty whether or not the actual velocity was changed.

Lucas (22) found that tension prolonged the time of contraction of a frog gastrocnemius muscle.

Method. In our experiments four kinds of muscle were used; the gastrocnemius, the sartorius, and the adductor magnus of the frog and the retrahens capitis collique of the turtle. In every case the muscle was set up in a moist chamber similar to that designed by Lucas (23), and described in a previous communication (24). The proximal end of each muscle was secured to a rigid stand. In the case of the gastrocnemius the knee joint was pinned to the stand (as in the experiments just cited (24)); in the case of the other muscles a thread was tied tightly around the muscle, and a pin was driven through the tissue so that the strain would come on the ligature when tension was applied, and the muscle would not be torn. A thread tied to the distal end in every case was drawn through a hole in the cover of the moist chamber and tied to the hook of a spring balance, the upper end of which was secured to a bar clamped rigidly to a stand. By raising this bar up and down the tension could be varied at will easily and rapidly.

For stimuli we used single break shocks from a Berne coil calibrated in accordance with Martin's scale (25) with an amalgamated copper and mercury key (26, p. 132) to break the primary circuit. In the case of the gastrocnemius and adductor muscles stimulation was applied to the sciatic nerve; in the case of each of the other two muscles stimulating electrodes were applied directly to the proximal end of the muscle itself. The action currents were led off by means of agar non-polarizable electrodes previously described (27, p. 115); they were connected with the muscle substance by means of twine moistened with Ringer solution, looped lightly around the muscle in such a way that there was no shift of contact during contraction. The proximal lead was placed near the middle of the muscle at its biggest point and the distal lead at the tendinous end. The muscle fibers were as far as possible uninjured throughout their length, and the action currents were

therefore diphasic. The responses were recorded by means of a string galvanometer, Hindle type, with an air gap of 1.5 mm. The string used for most of the experiments was of gilded quartz of 1.5 micra diameter with a resistance of 19,500 ohms. For three of the experiments on the gastrocnemius a string of 25,000 ohms resistance and 1.5 micra diameter was used. Photographic records were made with a camera described in a previous communication (28).

The procedure was to make the first record with no tension on the muscle, and to make successive observations as the tension was increased by steps. These records were interspersed with frequent control observations without tension; in the majority of cases the control observations were made in alternation with those under tension. The action currents were recorded immediately after the tension was adjusted. In each observation 5 or 6 maximal break shocks were applied in the course of a second or two.

Results. In all, our final records include five complete experiments with the gastrocnemius from which a considerable number of measurements can be made, and two more experiments which admit of comparison but not accurate measurements, four complete experiments with the frog's sartorius and two with the neck muscle of the turtle. Although the total number of experiments is not large, a good many individual action currents were recorded alternately with and without tension in each experiment, and the results were so consistent as to warrant definite conclusions. The results with the adductor muscle were not consistent, and we concluded that owing to the complexity of its structure there were variable factors so difficult of control as to render further experiments with it unprofitable.

With the gastrocnemius muscle, the result was essentially in conformity with those of previous observers. The action currents were conspicuously larger when the muscle was under tension than when it was not. The excursions of the string increased in size as the tension was increased up to approximately 50 grams, at which a maximum was obtained. With further increase of tension the size of the excursions decreased in some instances, and in others remained the same. After tension had been increased to about 100 or 200 grams the size of action current appearing in the control observations interspersed between those under tension showed a decrease which became marked after further increase of tension; after such tension as this had been applied, the responses under tension began to decrease, yet continued to be greater than the controls.

A typical experiment is shown in table 1. The order in which the observations were made appears in the first column; in the second are shown the excursions when the muscle was not under tension; the third gives the tension applied, and the corresponding excursions are in the fourth column. Each figure given shows the average of several measurements of a series of separate action currents. Figure 1 shows typical galvanometric records of action currents before, during and after the application of tension. The figure shows both the increase under tension and the decrease in the control after strong tension.

TABLE 1

Gastrocnemius

	EXCURSION WITHOUT TENSION	TENSION	EXCURSION WITH TENSION
	mm.	*grams*	*mm.*
1	8.3		
2		10	10.5
3	8.4		
4		20	11.1
5	8.3		
6		50	10.9
7	7.8		
8		80	10.8
9	8.4		
10		100	10.6
11	7.8		
12		150	10.6
13	7.9		
14		200	10.5
15	7.1		

With both the frog's sartorius and the neck muscle of the turtle, there was no increase in action current correlated with tension, such as was found with the gastrocnemius. On the contrary, there was a slight tendency toward decrease, although with moderate tension this change was neither marked nor constant. When the tension became fairly great, the excursions in both the control records and those taken with tension showed a marked decrease. The results of a complete experiment on each muscle are given in tables 2 and 3. Typical records before, during and after moderate tension are shown in figure 2.

From the above facts it appears that a common feature of all the muscles studied is the decrease in control action currents after tension greater than a certain amount has been applied. This decrease per-

sisting after tension is withdrawn suggests damage to the fibers. In the experiments on the sartorius the responses under tension corresponded closely with those made immediately afterwards without tension. In other words, there was little effect produced by tension other than the permanent reduction of response, this being about the same size whether the tension was maintained or withdrawn, and becoming very small after tension had gone beyond a certain point. In one experiment on the neck muscle of the turtle a marked diminution of response under tension appeared before the permanent reduction (persisting after withdrawal of tension) had occurred, but soon the responses became very small whether the muscle was stretched or not.

A B C D E

Fig. 1

A B C

Fig. 2

Fig. 1. Galvanometric records of diphasic action currents, gastrocnemius muscle; shown in the order in which they were made. Stimulus in all: break shock, 147 Z units applied to sciatic nerve.

Gilded quartz string, 1.5 micra diameter, 25,000 ohms, damped with 0.1 mf. condenser in parallel. String tension in all, 41 meters per amp. (See Forbes and Ray: This Journal, 1923, lxiv, 435.) Magnification, 340.

The first phase of the action current, from which measurements were made, appears as a sharp rise in each record.

Time shown below by tuning-fork shadow, 100 d. v. per second.

Tension on muscle as follows:—*A*, 0; *B*, 50 grams; *C*, 0; *D*, 200 grams; *E*, 0.

Fig. 2. Records of diphasic action currents; *S*, sartorius muscle of frog; *R*, retrahens capitis collique of turtle; shown in order as made.

Gilded quartz string, 1.5 micra diameter, 19,500 ohms; string tension, 79 m. per amp. Magnification, 490.

Stimuli: in *S*, break shocks, 472 Z units; in *R*, break shocks, 646 Z units. In both, stimuli applied directly to muscle.

Tension on muscle: In *S:* *A*, 0; *B*, 30 grams; *C*, 0. In *R:* *A*, 0; *B*, 40 grams; *C*, 0.

In the case of the gastrocnemius the decline of the maximum response with increasing tension was correlated with a decline in the size of response in the control records without tension. In short, it appears that with all three types of muscle studied, tension beyond a certain point impairs the fibers and reduces the size of action current of which they are capable (cf. 29). In the turtle's neck muscle and in the frog's sartorius this seems to be the only effect; whereas, in the frog's gastrocnemius there is, before the tension has sufficed to cause this

TABLE 2

Sartorius

	EXCURSION WITHOUT TENSION	TENSION	EXCURSION WITH TENSION
	mm.	*grams*	*mm.*
1	12.3		
2		1.0	12.0
3		2.8	12.0
4	12.0		
5		3.8	12.4
6		5.5	12.2
7	11.2		
8		8.3	11.6
9	10.7		
10		10.0	10.3
11	10.0		
12		20.0	9.0
13	8.3		
14		40.0	6.9
15	6.8		
16		60.0	6.2
17	5.7		
18		70.0	5.7
19	5.7		
20		80.0	5.5
21	4.6		
22		100.0	3.9

impairment, an increase in the size of action current correlated with the amount of tension being applied at the moment, but leaving no after-effect.

The most probable clue to the interpretation of these results seems to lie in the topographical arrangement of the fibers in the different muscles. In the frog's sartorius and in the neck muscle of the turtle, the fibers are parallel to the long axis of the muscle. In the frog's gastrocnemius the fibers are oblique to the long axis of the muscle as

a whole, converging upon a tendon which occupies a somewhat central position. This latter arrangement makes possible a change in the relation of adjacent fibers to each other when the muscle is stretched.

Since the two muscles in which the fibers show the simple, parallel arrangement both behave in the same way under tension, showing little or no change in action current other than that which appears to signify damage, it seems reasonable to look to the peculiar arrangement of the fibers of the gastrocnemius for the explanation of the increase in its action current under tension.

TABLE 3

Turtle muscle

	EXCURSION WITHOUT TENSION	TENSION	EXCURSION WITH TENSION
	mm.	*grams*	*mm.*
1	4.2		
2		20	3.8
3	3.6		
4		20	3.2
5		30	3.1
6	3.2		
7		30	3.0
8		40	2.9
9	3.1		
10	3.3		
11		40	2.8
12		50	2.8
13	3.0		
14		50	2.2
15		60	2.1
16	2.7		
17		70	1.3
18	3.7		
19		40	1.3
20	3.5		
21		70	1.1
22	1.3		
23		30	1.1

Let us consider a probable effect of stretching on the action current of fibers arranged as they are in the gastrocnemius muscle. Figure 3 shows in diagrammatic and exaggerated form the significant feature of their arrangement. When no tension is applied, the fibers will make the greatest angle with the long axis of the muscle (as in *C*); when the muscle is stretched the fibers will be elongated and will lie

more nearly parallel with the muscle's long axis, *B*, and the overlapping of adjacent fibers will become relatively less. The extreme conditions between which *B* and *C* (representing actual conditions) are intermediate, are shown in *A* and *D*. In *A* the fibers are parallel with the

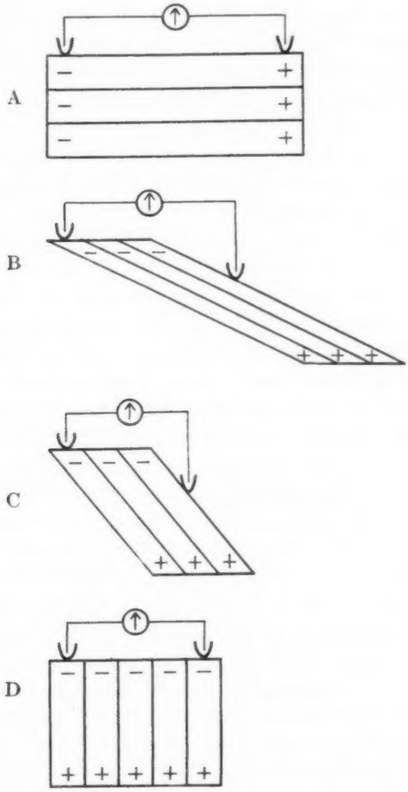

Fig. 3. Diagrams showing effect of topographical arrangement of fibers on recorded action current. *A* shows parallel arrangement giving maximum current in galvanometer. *B* and *C* illustrate oblique arrangement similar to that in gastrocnemius; *B*, under tension; *C*, without tension. *D* shows extreme case in which fibers are symmetrical with respect to leads, and no current will appear in the recording circuit.

long axis of the muscle. If the propagated disturbance starts simultaneously at corresponding points in all the fibers, this arrangement will be most favorable for registering an action current, for the points of maximum activity (and therefore of maximum electrical negativity) in all will be directly under the proximal electrode at the same time. If the fibers are oblique and converge so sharply on the tendon that there is pronounced overlapping (e.g., as in *C*) and if again the disturbance starts simultaneously at corresponding points, there will be a certain amount of interference between the action currents of individual fibers. At the moment when that part of some fibers lying directly under the proximal electrode is negative, the more distal portion of other fibers, also directly under the electrode, will be positive, and their effect will be to reduce the resulting current derived in the galvanometer circuit. This point may perhaps be made clearer by considering the extreme case, shown in *D*, in which the fibers are set at right angles to the axis of the muscle. In this case no current would appear in the galvanometer circuit. As the muscle is stretched, the arrangement of the fibers changes in a manner similar to the change from *C* to *B*. As the arrangement thus approaches more nearly to that shown in *A* the action currents derived in the galvanometer circuit become larger. Although the gastrocnemius muscle does not present the geometrical simplicity of the diagrams, the same principle probably applies.

In support of this explanation we may refer to the experiments of Samojloff (15) already cited, in which he showed a minimum in the action current of the gastrocnemius correlated with the time of greatest shortening following a previous propagated disturbance, the minimum being present in the isotonic, but absent in the isometric contraction. In the isotonic contraction of the muscle the shortening of the fibers must increase the angle they make with the long axis of the muscle, and would therefore, according to our explanation, tend to reduce the apparent size of the action current. In the isometric contraction, when shortening is prevented, the obliquity must undergo little or no change, and this might well explain the absence of the decrease in action current under this condition.

An attempt was made to verify this supposition by means of the rectus abdominis muscle of a frog. This was dissected out and fastened to two rigid sticks in the manner of a square sail. We tried to determine the effect of obliquity in the fibers on the resulting action current. But we were unable to maintain sufficiently constant contact of either stimulating electrodes or leads to enable us to obtain even approximately consistent results, and the attempt was soon abandoned.

SUMMARY

1. In the gastrocnemius muscle of the frog, the action currents in response to single stimuli are distinctly greater when the muscle is stretched with moderate tension than when it is not.

2. In the frog sartorius and the neck retractor of the turtle, similar tension causes no definite change in action current.

3. All three muscles when stretched beyond a certain amount show a reduction in the size of action current whether the tension is being applied at the moment or not. This effect seems to denote a more or less permanent damage to the fibers.

4. The increase in action current under moderate tension, found in the gastrocnemius and not in the parallel-fibred muscles, can probably be explained by electrical interference resulting from the oblique or converging arrangement of the fibers in the case of the gastrocnemius muscle.

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HEMOLYTIC ACTION OF RADIUM EMANATION

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The ability of radiations from radium to hemolyze erythrocytes has been observed by Henri and Mayer (1), Chambers and Russ (2), Solmanson (3) and Hausmann (4). It has been the purpose of this investigation to study the process in some detail, in the hope of throwing light upon the destruction of protoplasm by these rays. It must not be inferred that the phenomona here described occur during the clinical application of radium, as it is improbable that the erythrocytes circulating through a radiated region are ever subjected to such heavy doses of rays as we have employed.

Chambers and Russ (1) obtained hemolysis only when human blood was exposed to α-rays; the β- and γ-rays being without effect. Hausmann (4) on the other hand has hemolyzed rabbits' corpuscles with β-rays. We have found that sheep's blood placed in contact with a glass tube containing radium emanation becomes laky, first about the walls of the tube, later throughout its entire mass. Inasmuch as the glass walls of the tubes were sufficiently thick to exclude all α-rays, there can be no question that the combined β- and γ-radiation can liberate hemoglobin from the erythrocyte. Whether the γ-rays alone can produce hemolysis has not been demonstrated. Experiments of Bergonie and Tribondeau (5), who exposed defibrinated blood to the action of x-rays, yielded negative results, but longer and more intense exposure might have produced hemolysis.

In the majority of our experiments radium emanation has been in solution in the blood so that all three sorts of radiation have been brought to bear on the corpuscles. Table 1 indicates that the combined hemolytic effect of the α-, β-, and γ-rays from 0.46 millicurie of emanation in solution is equivalent to that of the β- and γ-rays given off by a glass tube containing 54 millicuries. Exclusion of the α-radiation by the tube decreases the hemolytic effect a hundred fold, which is of the magnitude to be expected if the hemolytic power is closely related to the

ionization produced by the rays. The conditions under which these determinations were made were not sufficiently comparable to justify any conclusion concerning the relation of hemolysis and ionization produced by the different types of ray, but they do warrant the deduction that the hemolytic activity of radium emanation when in solution is due almost entirely to the α-rays.

Corpuscles from defibrinated sheep blood, freshly obtained from the slaughter house, have been used exclusively. Possible complications arising from the action of the rays upon the serum have been avoided by washing the corpuscles three times in 0.9 per cent sodium chloride and then suspending 8 parts of the concentrated corpuscles in 12 parts of this salt solution. This ratio of corpuscles to salt solution was selected as being most favorable for the colorimetric method employed in measuring the rate of liberation of the hemoglobin.

TABLE 1

DATE	MILLI-CURIES	EXPOSED TO	TIME	PER CENT HEMOLYSIS
			hours	
March 26, 1918	54.0	β- and γ-rays	5	25
April 16, 1918	0.46	α-, β- and γ-rays	5	25

In order to get the emanation into solution, glass vessels of a shape suitable to the measurements for which they were to be employed were prepared. These were sealed on to the apparatus for purifying emanation described by Duane (6) by means of a tube drawn out into a capillary, were exhausted and a small quantity of emanation admitted into them. The vessels were then sealed off and allowed to stand over night to enable the decomposition products of the emanation to reach an equilibrium, and then the quantity of emanation was measured by means of the method and apparatus of Duane. To fill these vessels with blood it was only necessary to break the tip of the capillary tube, through which they had been exhausted, under the surface of the fluid and allow the negative pressure in the vessel to draw in the blood. The capillary tip was then sealed off with paraffin and dipped into mercury to avoid loss of any of the emanation.

Hemochromolysis. The liberation of the blood pigment, or hemochromolysis, has been measured by comparing the color of the tube containing the radiated corpuscles with a series of tubes containing unhemolyzed corpuscles diluted with various proportions of corpuscles hemolyzed by freezing. Such a standard series is sufficiently stable,

throughout the time of a single experiment, provided the precaution is taken to keep it on ice. Under the conditions of the experiments comparison could be made with this series with an accuracy of 5 per cent, and considerably greater accuracy was obtainable between 30 and 80 per cent hemochromolysis. The method allows the course of the reaction to be followed continuously.

Curve *A* in figure 1 illustrates the way hemochromolysis proceeds as a function of time. It may be seen that the velocity at which hemoglobin is liberated increases steadily until over half the hemoglobin is liberated. During the latter half of the process the rate of hemochromolysis is almost uniform in many experiments, in others it declines slightly after 80 per cent of the pigment is liberated.

Stromatolysis. The destruction of the stromata of the red corpuscle, or stromatolysis, as the result of radium radiations, may be watched directly under the microscope if a glass tube containing radium emanation is placed in a large drop of blood and a coverslip carefully floated upon it. By gauging the quantity of blood properly a preparation is obtained in which only a thin layer of corpuscles separates the tube from the coverslip. As the corpuscles settle most of them slip down around the side of the tube leaving only a few isolated blood cells resting upon its upper surface and these can be observed distinctly. With a tube of 40 millicuries of radium emanation no change can be detected in the corpuscles during an exposure of 1½ hours. Hemochromolysis must be proceeding during this time as the blood surrounding the tube becomes laky. At the end of this period stromatolysis takes place. The corpuscle under observation suddenly becomes indistinct and slowly fades away, as though going into solution. Twenty seconds after the process begins it is completed and the cell has disappeared.

Such observations suggest a distinct difference in the dynamics of hemochromolysis and stromatolysis. Whereas hemochromolysis appears to begin almost at once on exposure to radium and to proceed gradually, stromatolysis is a sudden change which occurs after a long period of exposure. To test this point further a quantitative comparison was made of the degree of hemochromolysis and of stromatolysis in the same suspension of corpuscles. Samples were drawn into a number of tubes, each containing a like quantity of radium emanation, and as hemolysis proceeded these tubes were compared with a standard series to measure the per cent of hemoglobin liberated. In order to measure the degree of stromatolysis a tube was opened from time to time and a count made of the number of corpuscles remaining intact. Table 2 summarizes such an experiment.

It is obvious that stromatolysis lags far behind hemochromolysis and that large quantities of hemoglobin can be liberated before destruction of the stromata occurs.

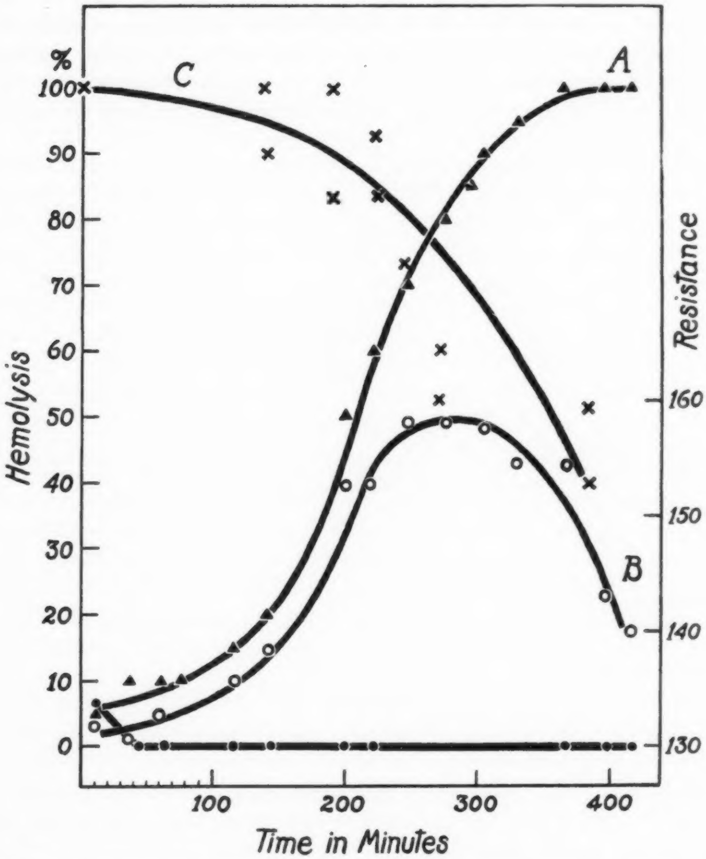

Fig. 1. Hemolysis by radium emanation. *A*, hemochromolysis; *B*, specific electrical resistance; *C*, stromatolysis; *D*, specific electrical resistance of unradiated control.

Changes in the electrical conductivity of corpuscles. Stewart has shown that the red blood cell is a very poor conductor of electricity when it is intact (7). Upon hemolysis the conductivity of a suspension

of corpuscles is altered in a direction and degree which depends upon the nature and violence of the hemolytic agent employed. Distilled water, ether and strong solutions of saponin, when used as hemolytic agents, enable the intra-corpuscular electrolytes to contribute to the conductivity of the blood so that the hemolyzed sample has its conductivity increased above normal; blood laked by freezing and by gentle heating is little changed in its resistance to the passage of an electric current. Blood hemolyzed by radiations from radium may be classed with the

TABLE 2

PER CENT HEMOLYSIS	NUMBER OF RED CORPUSCLES PER CU. MM.	PER CENT STROMATOLYSIS
0	14,800,000	0
20	13,300,000	10
40	12,400,000	16
60	13,800,000	7
70	10,700,000	28
80	8,900,000	40
100	7,700,000	48

TABLE 3

Conductivities of suspensions of sheep corpuscles in 0.9 per cent NaCl solution before and after hemolysis by radium emanation

	MAY 14	MAY 30	JUNE 6
	I	II	III
Radiated sample:			
1. Before radiation	9.80×10^{-3}	7.54×10^{-3}	6.98×10^{-3}
2. At completion of hemolysis	8.37×10^{-3}	6.83×10^{-3}	6.23×10^{-3}
Control sample:			
3. Before radiation	9.47×10^{-3}	7.50×10^{-3}	7.09×10^{-3}
4. At time hemolysis was complete in radiated sample	9.39×10^{-3}	7.53×10^{-3}	7.12×10^{-3}

latter group as its conductivity is slightly decreased by the process of laking. Table 3 presents the conductivities of several samples of blood before and after radium hemolysis had occurred.

It is instructive to follow the changes in conductivity as hemolysis occurs and to correlate them with the amounts of hemochromolysis and stromatolysis. Conductivity cells of the pipette type have been constructed which could be filled with radium emanation and corpuscles in the manner described on a preceding page. As hemolysis proceeded measurements were made from time to time of the conductivity of the

sample and the corresponding degree of hemochromolysis was determined colorimetrically. Throughout the experiment the cell containing the corpuscles was kept at a temperature of 25+ 0.1°C. and was shaken by a mechanical device in order to minimize the settling out of the corpuscles. In figure 1 data obtained from such an experiment are plotted. As the liberation of the blood pigment proceeds (curve *A*) the resistance of the sample (curve *B*) increases at a rate which is directly proportional to the rate of hemochromolysis. The close parallelism of the two curves ceases when a little over half the hemoglobin is liberated and the curve of resistance passes through a maximum and falls off as rapidly as it has arisen. Curve *C*, based upon the data in table 2, has been introduced into the figure in order to correlate the process of stromatolysis with these curves. This curve, which expresses the number of corpuscles remaining intact at any stage in the process, starts to fall off rapidly at just the time that the parallelism between hemochromolysis and resistance ceases.

These relations suggest the following explanation of the changes in conductivity of suspensions of erythrocytes. At the beginning of radiation hemoglobin is liberated from the corpuscles, which are themselves non-conductors, and this will, as Stewart has shown, depress the conductivity of the suspending salt solution. At this time the destruction of the stromata is negligible and the intracellular electrolytes do not escape in sufficient quantity to alter the conductivity of the sample. After hemochromolysis is about half completed the stromata commence to go into solution in rapidly increasing numbers and as they do so the contained electrolytes are able to take part in the conduction of the current in sufficient amounts to more than counterbalance the continued tendency of the liberated hemoglobin to increase the resistance. The data do not enable us to decide whether the intracorpuscular electrolytes contribute to the conductivity of the suspension in increasing amounts during the first part of the process or not, but if they do so there is, at just the time when the stromata commence to go to pieces in significant quantities, a critical change which enables the increased number of conducting ions to overcome the steadily growing resistance due to the liberation of larger and larger quantities of hemoglobin.

SUMMARY

The destruction of erythrocytes by radium emanation is due chiefly to the action of α-rays.

The processes of hemochromolysis and stromatolysis proceed independently of one another.

The electrical resistance of the suspension increases as hemochromolysis proceeds, and is reduced again when stromatolysis occurs.

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THE ADRENALS AND PANCREATIC DIABETES[1]

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It has been maintained by a number of writers that the presence of the adrenals is necessary for the development of pancreatic diabetes. The evidence brought forward in support of this contention is so weak that it is sometimes difficult to realize that it could have been seriously considered.

Zuelzer (1) tied the adrenal veins in a dog and extirpated the pancreas. Only a slight glycosuria (0.2 per cent of sugar) was found in the first urine obtained, and glycosuria did not develop in the 36 hours for which the animal lived. No blood sugar examination was made. Yet he concludes that pancreatic diabetes did not develop because the discharge of epinephrin from the adrenals was stopped. He finds support in the experiment for his theory of the mutual antagonism of the internal secretion of the adrenal (epinephrin) and the internal secretion of the pancreas. Pancreatic diabetes, according to him, is an adrenalin diabetes, due to the unchecked action of the epinephrin physiologically secreted by the adrenals in the absence of the antagonistic pancreatic hormone. In favor of this view he emphasizes his observation that a pancreatic extract prepared by him is capable of suppressing adrenalin diabetes (produced by artificial injection of adrenalin). To show how baseless this theory is, it is sufficient to point out that pancreatic extracts (insulin), as since shown by Macleod and his collaborators (2), will prevent the development not only of adrenalin hyperglycemia, but of the hyperglycemia caused by mechanical asphyxia, carbon monoxide, ether and piqûre. We (3) have found that the same is true of morphine hyperglycemia. So that there is nothing specific in the effect of the pancreatic internal secretion upon the adrenalin diabetes. Further, according to the available data, the amount of epinephrin given off from the adrenals is far inferior to that required for the development of

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adrenalin diabetes, and the output is stated to be diminished rather than increased after pancreatectomy (4).

Mayer (5) failed to answer the question whether preliminary extirpation of the adrenals (in dogs) modifies the diabetes which follows ablation of the pancreas, because all the animals died either during the operation or soon thereafter. When he approached the problem from the other side, removing first the pancreas and then the adrenals, he was also unsuccessful in getting definite information. Out of 11 dogs from which the pancreas was removed, and after an interval of 2 hours the adrenals, only 3 survived for 1 hour. Although there is no doubt that the operation, as practised in this way, is rapidly fatal, this extremely bad result indicates that the surgical technique was inadequate. In 6 cats the operation was performed piecemeal. The right adrenal and the head of the pancreas were first excised, and a month later the rest of the pancreas. Three of the cats died at this stage. The remaining 3 developed glycosuria. After 8 days the left adrenal was removed, and the cats died in 2, 4½ and 5 hours respectively. Because the rate of urinary secretion and the percentage of sugar in the urine (blood sugar was not determined) in these dying animals, suffering not only from adrenal deficiency but from the shock of the operation, were somewhat less than before the removal of the second adrenal, when they were suffering simply from pancreatic diabetes and were certainly not dying, he draws the conclusion that removal of the adrenals seems to influence the pancreatic glycosuria. Even although he qualifies the statement by saying that the short survival of the animals "ne permet pas d'être très affirmatif," it is incomprehensible how any value whatever should have been given to such results. How is it possible, for instance, to say that the rate of urinary secretion was diminished by the loss of the second adrenal because after the operation, in the 2 hours for which the animal lived, only 4 cc. were collected (i.e., at the rate of 48 cc. for the 24 hours), as compared with 115 cc. in the preceding 24 hours? It is astonishing rather that so much was secreted in a dying animal, and it is likely that the rate must have been higher in the first part of the 2 hours than later on. It is in any case quite futile to draw a quantitative conclusion from such data.

Frouin (6) removed one adrenal and about ⅔ of the other from 2 dogs (in 2 operations). A month after the second operation he commenced the ablation of the pancreas, which was completed in 2 operations, with an interval of 2 months between them. One dog lived 16 days, the other 25 days. No blood sugar estimations were made. The per-

centage of sugar in the urine was determined. But if we interpret his statement correctly, the urine was not collected for the whole 24 hours, but only from 8 a.m. to 8 p.m. Not enough data are given to show how frequently the sugar was determined. He concludes that the volume of urine secreted, as well as the amount of sugar excreted, was diminished, or, as he puts it, the intensity of the pancreatic diabetes was diminished by the partial removal of the adrenal tissue. But this conclusion is reached simply by comparing the percentage in the urine and total amount of sugar excreted in 12 hours in these 2 dogs (3.0 to 1.7 per cent and 2.3 grams of sugar; 3.1 per cent and 5.1 grams of sugar respectively) with what is said often to be seen in dogs after ablation of the pancreas alone (10 per cent and 20 to 30 grams in 24 hours). No control animals observed in the investigation are mentioned, nor is it stated that the food and drink were in any way controlled. The statement that the quantity of urine excreted was diminished (mean of the 12 hours, 130 cc. and 165 cc. in two dogs, weighing 20 and 23 kgm. respectively) has therefore no significance. No results on the same animals prior to the last operation are mentioned. All that is said is that in simple pancreatic diabetes "on ne constate pas de diminution du volume de l'urine. Certains auteurs ont même observé de la polyurie." Our own experiments show that after pancreatectomy in dogs whose epinephrin output has been suppressed, by operations practised by us, the percentage of sugar in the urine is of the same order as in dogs which have undergone pancreatectomy alone. In our opinion Frouin's two experiments, as well as those of Mayer and Zuelzer on this subject, prove nothing at all, and should be written off.

Hédon and Giraud (7) and Hédon (8), to reduce surgical shock to the minimum, extirpated the adrenals, either both or the left (the right having been excised by a prior operation), in dogs previously depancreatized except for a portion which was dislocated under the skin. The animals were allowed to recover after the first operation on the pancreas, and had no glycosuria. The pancreatic remnant was extirpated immediately after completion of the adrenalectomy. The blood sugar was determined by Bang's micro-method. Either no increase was seen or only a slight one, in the period of survival. Eventually before death the blood sugar sank below the value prior to complete removal of the pancreas. Hédon and Giraud conclude that a functional relation appears to exist between the pancreas and the adrenals for the metabolism of sugar. But they explain that this does not in any way commit them to the support of Zuelzer's theory (of an antagonism between the

internal secretion of the pancreas and adrenalin). The weak point in these experiments, although they are much superior to those previously alluded to, is that even when the operations are performed in stages, the total removal of adrenal tissue in dogs is compatible with only a short period of survival, which is probably curtailed still more by total removal of the pancreas. While we interpret them differently, we have had precisely the same results as Hédon and Giraud in dogs which only survived for a short time total pancreatectomy and removal of the left adrenal, although the animal had recovered from a preliminary operation, in which the right adrenal was excised and the left gland denervated, with destruction of its medulla by curetting with a drill.

Thus, in dog 833, a female, weighing 8.05 kgm., the right adrenal was excised, the left denervated and the medulla curetted. The blood sugar content, 39 days thereafter, was 0.083 per cent at 4:00 p.m. The dog was in fair condition, but had lost weight. On the next day the pancreas and the left adrenal were removed, the operation being completed at 11:00 a.m. At 4:00 p.m. the blood sugar was 0.087 per cent, that is, a hyperglycemia had not developed in the first 5 hours following total pancreatectomy. The temperature was 39.9°, pulse 168, respiration 40. The animal died in the following night, so that a further blood sugar determination was not made. The urine (21 cc.) voided since 5:00 p.m. on the day of the pancreatectomy contained 0.49 per cent of sugar.

In all our experiments in this paper the blood sugar was estimated by the Folin-Wu method and the sugar in the urine by the Shaffer-Hartmann method.

In another dog (839), a young adult female, weighing 7.8 kgm., the right adrenal was excised, the left denervated and the medulla curetted. The blood sugar, 35 days thereafter, was 0.089 per cent. On the 36th day after the first operation the left adrenal was excised, and pancreatectomy completed at 11:18 a.m. At 4:00 p.m. the animal refused to take either meat or milk. The dog passed in the night 770 cc. of urine, which contained 0.14 per cent sugar. About 60 cc. were voided up to 3:00 p.m. on the day following removal of the pancreas. It contained 0.18 per cent of sugar. At 4:00 p.m. on that day the temperature was 37.9°; pulse 120; respiration 60; blood sugar 0.076 per cent. The animal died at 5:45 p.m., 30½ hours after the final operation.

As already remarked, we do not think the absence of a hyperglycemia in these moribund animals has any special signifiance. It may be quite true, as Hédon and Giraud say (9), that in a dog whose adrenals have not been interfered with, or in a dog after partial removal of the adrenals, the sugar content of the blood begins to rise decidedly in a time much shorter than the common survival period in their experiments with combined pancreatectomy and total adrenalectomy. But where an animal is going to die in 7 or 8 hours, it would surely be very risky to assume that there were any of these hours in which it was not dying,

and that the reason for the failure of diabetes to appear in the absence of the pancreas was due to the absence of the adrenals as such, and not to the inability of the dying organism to develop a hyperglycemia. As a matter of fact, in the dog which survived the longest (about 30 hours) in Hédon and Giraud's experiments there was some tendency to an increase in the blood sugar. There is nothing specific in the fact that before death these animals may show a hypoglycemia. In various other conditions in which neither the pancreas nor the adrenals have been interfered with, a hypoglycemia may develop when the animal is dying.

Hédon and Giraud found that in a dog in which pancreatic diabetes had already been established after total pancreatectomy, removal of the adrenals did not at all diminish the diabetes during the first 8 hours, either for the hyperglycemia, which was maintained at 0.3 per cent, or for the excretion of sugar. Only later did the blood sugar diminish, to 0.22 per cent at the 23rd hour, and 0.17 per cent at death (25th hour). Strangely enough, they do not consider that this persistence of the pancreatic diabetes after removal of the adrenals conflicts with the view that the adrenals are essentially concerned in the diabetes. For, say they, it is not surprising that "the attenuating effect of adrenalectomy is tardy when one knows how resistant pancreatic diabetes is to influences logically the most proper to enfeeble it." We cannot help thinking, however, that a hyperglycemia which is still but slightly diminished 23 hours after removal of the adrenals, and is quite distinct at the time of death, cannot have depended essentially on any action of the adrenals. And one might just as well say that it is not surprising that the diabetic effect of pancreatectomy is tardy in dogs whose adrenals have been simultaneously removed, when one knows how profound and acute is the depression caused by this operation. It seems probable that if the survival period could be prolonged, the diabetes would develop in spite of the absence of the adrenals.

In criticising previous work on this subject, it was pointed out by us (10) that "As it is the epinephrin given off from the adrenals which has always been considered the important thing in these theories, a much better experiment could be made upon dogs which had recovered completely after an operation for suppression of the epinephrin output." For the pancreatectomy can then be carried out on animals which are probably as good operative risks as normal animals. In our experiments suppression of the epinephrin output was accomplished by removal of one adrenal and denervation of the other, with destruction of the medulla of the second adrenal by curetting. Some of the cortex is,

of course, also destroyed in curetting sufficiently to insure destruction of the medulla. This is no detriment but rather an advantage, for the purpose of the experiments in question, so long as an amount of cortex sufficient to permit survival is spared. In addition, to prevent hemorrhage, one-fourth to one-third of the demedullated adrenal was usually tied off by a ligature, placed generally around the upper pole where the drill was inserted, so that not even the whole of the cortex of the second adrenal remained. The damaged adrenal was preserved for microscopic examination.

Less than a year afterwards Houssay and Lewis (11) published a note containing the results of experiments on 5 dogs, in which they curetted away the medulla of the left adrenal and extirpated the right. After about a month the pancreas was removed. Three of the dogs did not develop any hyperglycemia, or showed rather a hypoglycemia. One of these animals died of infection (peritonitis) 4 days after the pancreatectomy. Two others only survived this operation 6 and 24 hours respectively. The remaining 2 animals lived only 8 and 3 days, respectively, after removal of the pancreas, and showed decided hyperglycemia and glycosuria. But in only one of these cases was the experiment considered free from flaw, since microscopic examination of the left adrenal in one of the dogs revealed the presence of a small remnant of undestroyed medullary tissue. This leads us to remark that our method is superior, since the denervation alone practically abolishes the epinephrin output, and the curetting of the medulla is an added precaution. In our experience such a short average period of survival after pancreatectomy of dogs with an ample residue of adrenal cortex is not inherent in the operation, when performed with adequate surgical technique.

We have by our method obtained unequivocal evidence that the absence of epinephrin does not hinder the development of pancreatic diabetes. We could observe no essential difference in the symptoms, in the degree of hyperglycemia, its rapidity of onset and persistence and in the percentage of sugar in the urine in dogs prepared in this way and in dogs whose adrenals had not been interfered with. It was shown also that when the remaining (already denervated and demedullated) adrenal was removed, the diabetes persisted, although when death was approaching the blood sugar might diminish.

The pancreas was always removed at one operation. The abdominal incision was made in the median line, or about 15 to 20 mm. to the right of it, and was about 75 to 100 mm. in length. The intestines were prevented from obstructing

the operative field by gauze packs. The head of the pancreas, with the duodenum, was brought up and held in view by moderate traction on two bands of tape, which were passed between the duodenum and pancreas just above and below the head of the pancreas The pancreatico-duodenal vessels were exposed by splitting the mesenteric folds and pancreatic tissue by blunt dissection, and the head of the pancreas tied off with heavy silk ligatures, placed so as not to interfere with the vessels as they go into the pancreatic tissue. This aids in controlling hemorrhage during the separation of the head from the duodenum. The pancreatic branches of these vessels were now carefully isolated, and nearly all of them tied separately with thin silk ligatures. In some instances only the larger branches were tied; the smaller ones were caught in a hemostat and twisted until torn out of the pancreatic tissue (this was usually done in small young dogs and did not result in much bleeding). With the aid of a sharp curette all the pancreatic tissue can usually be scraped away from the duodenum and the head of the pancreas separated with very little damage to duodenal branches of the pancreatic-duodenal vessels. The pancreatic ducts were cleaned of pancreatic tissue by curetting, tied off and the head separated from the duodenum. The duodenum was then wrapped in sponges, moistened with warm saline, and the body of the pancreas freed by splitting the mesentery and ligating small vessels. The tail was freed in a similar manner, and the gland completely removed. The duodenum was then surrounded by the omentum and replaced in the abdomen, and the wound closed. Metal retractors were not used, as they are liable to cause excessive traumatization. Healing of the wound by first intention was practically always obtained (except in one case where stitch infection was caused by slipping of the bandage and scratching of the wound by the animal). Healing did not seem to be more difficult after total pancreatectomy than after any other major abdominal operation, so that we saw no advantage from the surgical point of view in performing the operation in two stages, as recommended by Hédon. In none of our animals was there any evidence of material interference with the circulation of the duodenum. The animals were fed with lean meat and water ad libitum. They developed the typical progressive emaciation in spite of the large consumption of food, eaten greedily.

As illustrated in the following protocol (dog 834), the blood sugar rose from 0.096 per cent before pancreatectomy to 0.198 per cent, $5\frac{1}{2}$ hours after completion of that operation. Two days later it was 0.286 per cent, and it was maintained at about this level thereafter. The percentage of sugar in the urine collected in the metabolism cage overnight, i.e., for a period of 12 to 15 hours was 9 to over 10 per cent in several of the samples. This contrasts strongly with the relatively small percentages obtained by Frouin in his two dogs after complete adrenalectomy and pancreatectomy, and shows how risky it is to draw conclusions as to the question at issue from observations on moribund animals.

Condensed protocol. Dog 834. Female. Weight 8.15 kgm. Right adrenal excised, left adrenal denervated and the medulla destroyed by curetting. Total

pancreatectomy was performed 51 days thereafter (on March 9), the animal weighing only 6.75 kgm., but in good health. The pancreatectomy was completed at 11:00 a.m. At 4:30 p.m. the blood sugar percentage was 0.198. On the day previous to the pancreatectomy (March 8) it was 0.096. On March 11 it was 0.286; on March 15, 0.278. The quantity of urine collected overnight, with the percentage of sugar for each night from March 9 to 10 to March 18 to 19 was as follows: (130 cc.) 6.38 per cent; (240 cc.) 9.36 per cent; (119 cc.) 4.16 per cent; (137 cc.) 7.50 per cent; (75 cc.) 6.50 per cent; (129 cc.) 9.04 per cent; (58 cc.) 10.40 per cent; (150 cc.) 9.00 per cent; (69 cc.) 4.16 per cent; (75 cc.) 3.52 per cent.

The animal died in the night of March 18 to 19. The body weight was then only 4.725 kgm. The appetite had been good from the time of the pancreatic operation, about 700 grams of beef having been consumed daily. But on March 17 and 18 it was observed that the dog did not eat. At no time did it drink a great deal of water. The necropsy verified the complete removal of the pancreas. No evidence of any necrosis of the duodenum. Very little abdominal fat. Lungs, liver, spleen normal in appearance. The left adrenal weighed 0.63 gram.

In the next experiment, on dog 832, a male, weighing 10.3 kgm., the operations were carried out in the same way. The right adrenal was excised, the left denervated and its medulla curetted by a drill. The pancreas was totally extirpated, 33 days thereafter (on January 15). The blood sugar just before removal of the pancreas was 0.091 per cent. Three days later it was 0.227 per cent. On January 25, 26 and 27, the urine contained 4.30, 4.75 and 5.85 per cent of sugar respectively. On January 29 at 9:00 a.m., the blood sugar was 0.286 per cent. The remaining (left) adrenal, whose epinephrin output had been abolished 47 days previously, was excised at 2:30 p.m. On January 30 at 9:00 a.m. (18½ hours after removal of the second adrenal) the blood sugar was still at its high level, 0.288 per cent. At 4:00 p.m. (25½ hours after excision of the adrenal) the blood sugar was still 0.216 per cent. On January 30 the animal began to show distinct signs of increasing weakness. It took practically no water that day. Only 15 cc. of urine were collected from 8:00 a.m. to 5:00 p.m., and the sugar percentage in it was 4.8. On January 31 the animal was noticeably weak, refused food and took very little water. The percentage of blood sugar on the afternoon of this day (48 hours after complete loss of adrenal tissue) had dropped to 0.08 per cent. The circulation was poor, the blood dark and the veins collapsed. The rectal temperature was 39.6°C., pulse 184 and respiration 44 a minute. On February 1 at 9:15 a.m., the animal was dying. The temperature was 39.8°C., the pulse 60 a minute. Blood obtained from the jugular contained only 0.054 per cent of sugar. The respiration stopped at 9:20 a.m., about 67 hours after removal of the second adrenal. During the night 32 cc. of urine had been passed, containing 0.68 per cent of sugar. The necropsy showed complete absence of the pancreas.

This experiment, while demonstrating clearly that the lack of epinephrin from the adrenals does not alter in any recognizable way the typical course of the diabetes resulting from ablation of the pancreas, shows also that in the absence of both cortex and medulla the pancreatic diabetes proceeds without change until the animal becomes moribund. Then indeed the percentage of sugar, both in the blood and in the urine,

diminishes markedly. As already pointed out, the animals observed by Frouin, Mayer and other investigators were in this condition.

We performed a number of experiments in which the right adrenal was first excised, then, after an interval for recovery of the animal, the pancreas was removed, and finally, after a further interval, the left adrenal was extirpated. The dogs survived only a short time the removal of the second adrenal.

In dog 847, a female weighing 3.55 kgm., the right adrenal was excised. After 18 days, the dog then weighing 2.93 kgm., the pancreas was completely removed. The urine passed next night (14 cc.) contained 9.68 per cent of sugar. Three days after the pancreatectomy, the blood sugar was 0.192 per cent (at 9:00 a.m. on March 27). At 6:30 p.m. the left adrenal was excised under light ether anesthesia. The operation only required 9 minutes from the time of the first incision. Including the time of shaving and cleaning up, the animal was only 20 minutes on the table. Shock was so entirely avoided that about 5 minutes after the operation the dog walked in its cage. Every precaution was taken to present loss of heat both during the operation and afterwards. Yet death occurred in the night between March 27 and March 28. The small quantity of urine passed during this night contained 10.68 per cent of sugar. The urine collected just before removal of the second adrenal contained 2.05 per cent of sugar.

In another dog (845), a female weighing 3.34 kgm., the right adrenal was excised, and after 10 days the pancreas. At the second operation the animal was in good condition and weighed 3.26 kgm. The urine was examined for sugar daily for the next 8 days, the percentages varying from 4.5 to 11.4. Ten days after pancreatectomy, the body weight being 2.395 kgm., the blood sugar, at 9:00 a.m. was 0.236 per cent. On this day the left adrenal was excised under light etherization, the animal being on the board from 10:05 to 10:28 a.m. At 4:30 p.m. (6 hours after loss of the second adrenal) the blood sugar was still 0.205 per cent. The animal was already getting weak in the hind legs, and the urine contained only 0.7 per cent of sugar. Death occurred during the night.

As the ether was only lightly given for about 20 minutes, it does not seem reasonable to attribute to the operation any important part of the hyperglycemia still present at least 6 hours after removal of the second adrenal. A control experiment was made on a normal dog, a female, weighing 5.75 kgm. The blood sugar before etherization was 0.088 per cent. Ether was then given and continued, so as to maintain surgical anesthesia, for 42 minutes, i.e., twice as long as in the removal of the second adrenal in dogs 845 and 847. Ten minutes after discontinuance of the ether the blood sugar was 0.192 per cent; $1\frac{1}{3}$ hours later, 0.133; 3 hours later ($4\frac{1}{2}$ hours after the ether was stopped) 0.089 per cent.

If the adrenals are essentially concerned in pancreatic diabetes through the antagonistic action of epinephrin and the internal secretion of the

pancreas, as Zuelzer supposed, it might be expected that insulin would act differently upon a diabetic dog which had undergone the adrenal operation described and upon a diabetic dog whose adrenals had not been interfered with. A comparison was accordingly made between the action of insulin on dog 832, in which the operation for the suppression of the epinephrin output of the adrenals had preceded the pancreatectomy, and on dog 831, in which pancreatectomy alone had been performed. In both cases the diabetes was well established. In both animals a marked reduction in the blood sugar percentage was effected by the insulin. The same preparation was used for the two animals, and the dose was approximately proportional to the body weight. The two experiments were made simultaneously in the same room, so that the external conditions were the same. It would be obviously impossible, in the present state of our knowledge, to attempt to take account of quantitative differences in the action of insulin on the blood sugar content. All we desire to point out is that a very marked reduction was produced in both animals (from 0.227 to 0.097 per cent in 3 hours, and to 0.063 per cent in $4\frac{3}{4}$ hours in dog 832; from 0.250 to 0.108 per cent in less than 3 hours, and to 0.093 per cent in 4 hours 40 minutes in dog 831).

Condensed protocol of insulin experiment on dog 832. Weight 9.15 kgm. Adrenal operation 36 days and total pancreatectomy 3 days, before the experiment.

9:20 a.m. Temperature 39.3°; pulse 148; respiration 64.
9:25 a.m. Blood sugar 0.227 per cent.
9:30 a.m. 0.4 cc. iletin (Lilly, marked 50 units per cc.) given subcutaneously.
10:35 a.m. Blood sugar 0.182 per cent; T. 39.25°; P. 148; R. 28.
12:30 p.m. Blood sugar 0.097 per cent; T. 39.4°; P. 146; R. 32. At 2:00 p.m. the dog drank water, and 5 minutes later vomited.
2:15 p.m. Blood sugar 0.063 per cent; T. 39.6°; P. 160; R. 32.
3:50 p.m. Blood sugar 0.063 per cent; T. 39.5°; P. 144; R. 48. The animal is panting. It was panting when brought down for the experiment, then the respiration was quiet for about 2 hours after insulin was given. At 4:30 p.m. the animal ate very little meat when it was offered. Urine collected about midnight gave a strongly positive test for sugar.

Condensed protocol of insulin experiment on dog 831, 9 days after total pancreatectomy. No adrenal operation. Weight 3.51 kgm.

9:05 a.m. T. 38.7°; P. 132; R. 20.
9:10 a.m. Blood sugar 0.250 per cent.
9:30 a.m. 0.2 cc. iletin (Lilly, marked 50 units per cc.) subcutaneously. Same specimen was used in dog 832.
10:30 a.m. Blood sugar 0.222 per cent; T. 38.6°C.; P. 120; R. 28.
12:20 p.m. Blood sugar 0.108 per cent.

2:10 p.m. T. 39.2°C; P. 136; R. 16. Blood sugar 0.093 per cent.
3:20 p.m. T. 39.0°; P. 148; R. 24.
4:30 p.m. Dog refused meat. The urine collected at 6:10 p.m. gave a faint (doubtful) reaction for sugar. Another specimen voided about midnight gave a moderate reaction, and next morning there was plenty of sugar in the urine.

As the influence of insulin in suppressing the hyperglycemia caused by morphine was being studied (3), one experiment on the influence of morphine on the pancreatic hyperglycemia was made on dog 831, two days after pancreatectomy. The animal was active and in good condition. We have shown that the adrenals intervene in some way in the development of morphine hyperglycemia (12). As insulin counteracts this form of experimental hyperglycemia, and the presence of the adrenals favors it, it might have been thought that in a pancreatectomized dog with intact adrenals, the hyperglycemia would be increased by morphine. If any definite effect was produced, however, in this experiment, there was a slight diminution in the blood sugar content. It must be recognized that with nearly 0.3 per cent of sugar already in the blood, it might have been impossible for the morphine to increase the hyperglycemia further.

Condensed protocol of experiment on the effect of morphine on the blood sugar in the pancreatectomized dog 831.
10:15 a.m. Pulse 100; respiration 28.
10:20 a.m. Blood sugar 0.275 per cent.
10:25 a.m. Morphine sulphate 25.0 mgm. (5.5 mgm. per kilo.) hypodermically.
10:32 a.m. Vomited. Respiration becoming rapid.
10:35 a.m. T. 38.26°; P. 80 (forcible); R. 200+.
10:40 a.m. Sleeps. Panting respiration.
12:00 m. Deeply under. 12:30 p.m., T. 37.1°; P. 84; R. 100.
12:40 p.m. Blood sugar 0.266 per cent. Dog very quiet; asleep.
2:10 p.m. T. 37.0°; P. 84; R. 76. Asleep but aroused more easily.
2:20 p.m. Blood sugar 0.242 per cent.
3:30 p.m. Asleep, but roused easily.
4:40 p.m. T. 37.58°; P. 80; R. 104. Awake but very drowsy. Blood sugar 0.250 per cent.

Dog 831, a female, survived total pancreatectomy almost 5 weeks. The day of the operation (January 9) it weighed 4.58 kgm. Two days thereafter the blood sugar was 0.275 per cent; a week later 0.250 per cent. On January 25 the urine contained 4.30 per cent of sugar. On January 27 the urine of the forenoon contained 3.0 per cent, and the urine collected during the afternoon 3.16 per cent. On January 28 there was only 1.32 per cent of sugar in the forenoon urine. On January 30 the blood sugar was 0.236 per cent; urine 4.1 per cent. For the next 12 days the quantities of urine voided in the night and the percentages of sugar were as follows: (68 cc.) 5.74; (26 cc.) 5.4; (35 cc.) 3.56; (60 cc.) 9.36; (56 cc.) 7.62;

(29 cc.) 9.9; (—) 4.64; (92 cc.) 7.64; (39 cc.) 7.04; (136 cc.) 7.64; (124 cc.) 6.32; (70 cc.) 4.28 per cent. On February 1 the blood sugar was 0.258 per cent; February 3, 0.286; February 6, 0.292; February 8, 0.426 per cent. The animal had now become weak and "wobbly" on its feet. The rectal temperature was 38.9°C., the pulse 92, respiration 28. These had varied but little throughout the period for which the animal survived. Death occurred in the night between February 11 and February 12. The blood, mostly serum, obtained by heart puncture in the morning, contained 0.21 per cent of sugar, the bladder urine 1.45 per cent. The necropsy showed that the pancreas had been completely removed.

SUMMARY

The right adrenal was removed in dogs, and the left adrenal denervated. In addition the medulla of the left gland was destroyed by curetting with a drill inserted at the upper pole. A portion of the cortex of the left adrenal was also destroyed. The epinephrin output was of course abolished by this operation. After an interval for recovery of the animals, total pancreatectomy was performed. Diabetes appeared precisely as in dogs whose adrenals had not been interfered with. The hyperglycemia and glycosuria were as quickly developed, reached as high a level, and were similarly affected by insulin. Even after removal of the (already denervated and demedullated) left adrenal, the diabetes persisted in undiminished intensity, so long as the animals remained in good condition. There is, therefore, no experimental basis for the theories which assign to the adrenals (particularly to the epinephrin physiologically liberated from them) a special relationship to the pancreas in the regulation of the carbohydrate metabolism and the blood sugar content, and in the genesis of the diabetes which follows pancreatectomy.

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THE EFFECT OF INSULIN UPON MORPHINE HYPERGLYCEMIA[1]

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It has been shown by Macleod and his collaborators (1) that insulin exerts an antagonistic action on the hyperglycemia caused by piqûre,[2] epinephrin, ether, mechanical and carbon monoxide asphyxia. It has been established by us (2) that the adrenals are not essentially concerned in piqûre, asphyxial or ether hyperglycemia. On the other hand, in the production of the hyperglycemia occasioned by morphine (in the cat, dog and rabbit) the adrenals seem to intervene in some way (3). In view of the various theories which have been put forward on the action of the adrenals upon metabolism, especially carbohydrate metabolism, and particularly on the supposed relations of the adrenals to the pancreas in this matter, it seemed not without interest to see how morphine hyperglycemia is affected by insulin. The observations show that there is no obvious difference in the effect of insulin upon this, and upon the other forms of experimental hyperglycemia so far investigated. In cats and rabbits the rise in the blood sugar, which would have been expected to develop with the doses of morphine employed, was quite effectively hindered by the administration of insulin some time before, some time after, or simultaneously with the administration of morphine. In illustration of this statement are cited the protocols of two rabbits (823 and 824), one of which received insulin an hour before morphine, and the other morphine a little more than an hour before insulin. The

[1] A note was published in Proc. Soc. Exper. Biol. Med., 1923, xx, 341.

[2] They state that they made the puncture through the cerebellum because of the danger of hemorrhage in exposing the fourth ventricle by Eckhard's method. We found Eckhard's method quite satisfactory, and had no trouble with hemorrhage. With a little practice it is usually unnecessary even to slit the occipito-atlantoid ligament before making the puncture, which can be accurately made through the membrane.

blood sugar was estimated by the Folin-Wu method. The two experiments were done simultaneously in the same room. The same specimen of iletin was used.

Condensed protocol of rabbit 823, a female, weighing 2.345 kgm.

9:30 a.m.	T. 39.41°; pulse 280 to 300; respiration 76 a minute.
9:35 a.m.	Blood sugar 0.115 per cent.
9:40 a.m.	4 units of iletin (Lilly) given hypodermically.
10:30 a.m.	T. 38.9°; P. 300 +; R. 68. Blood sugar (10:38 a.m.) 0.068 per cent.
10:40 a.m.	50.0 mgm. morphine sulphate hypodermically.
11:45 a.m.	T. 39.15°; P. 160; R. 64 (shallow). Anus relaxed.
11:52 a.m.	Blood sugar 0.052 per cent.
1:10 p.m.	T. 38.48°; P. 176; R. 62. Is somnolent. Blood sugar (1:12 p.m.) 0.071.
2:30 p.m.	T. 38.49°; P. 160; R. 100 (shallow). Is stupefied. Blood sugar 0.086 per cent.
3:20 p.m.	T. 38.94°; P. 250 +; R. 80. Not so stupefied. Blood sugar (3:23 p.m.) 0.085 per cent. Now produced asphyxia off and on for 10 minutes, beginning at 3:30 p. m.
3:40 p.m.	Blood sugar 0.127 per cent.

The liver weighed 72.0 grams and contained 5.1 per cent of glycogen.

Here it will be seen that the blood sugar was reduced from 0.115 to 0.068 per cent under the influence of insulin in the hour before morphine was given. The reduction went still farther (to 0.052 per cent, in the first $1\frac{1}{5}$ hours after morphine; $1\frac{1}{3}$ hours later the percentage had begun to rise slightly (0.071), but was still much below the initial value. Even $4\frac{3}{4}$ hours after the administration of morphine it was only 0.085 per cent, at a time when with morphine alone a hyperglycemia might have been long since expected, and when the crest would probably have been passed. A short period of asphyxia brought the blood sugar up, although not to the extent which would have been likely, considering the large glycogen store, if insulin had not been given. We have pointed out elsewhere (3) that there is some reason to suppose that morphine itself may render the asphyxia hyperglycemia less easy to obtain at this stage. It is worthy of note that the body temperature did not fall throughout the experiment, as is seen in rabbits under the influence of morphine alone, possibly because of the increased combustion of sugar caused by the insulin. Insulin, when given by itself, has been seen to cause some rise of body temperature, though not always. It was observed that the general symptoms of morphine, including the effect on the respiration, were evident, although its effect on the blood sugar did not develop.

In the next experiment to be cited (rabbit 824) the order of administration of the morphine and insulin was reversed, the morphine being given first, so as to give the morphine hyperglycemia a start, so to say, upon the insulin. In spite of this the minimum blood sugar was reached considerably sooner after the administration of insulin than in rabbit 823. It is true the initial blood sugar percentage was higher in the latter. Also, as Macleod and his fellow workers (4) have shown, there are great individual variations in normal rabbits in this respect when insulin is given alone. In rabbit 824 there was no hyperglycemia at any time throughout the experiment. Even asphyxia caused no rise in the blood sugar, although the glycogen store was good. The temperature reached a maximum of almost 1.5°C. above the initial temperature.

Condensed protocol. Rabbit 824. Male. Weight 2.46 kgm.

9:45 a.m.	T. 39.5°; P. 205 +; R. 60. Blood sugar (9:50 a.m.) 0.083 per cent.
9:52 a.m.	50.0 mgm. morphine sulphate hypodermically.
10:45 a.m.	T. 39.06°; P. 172; R. 40. Blood sugar (10:50 a.m.) 0.093 per cent.
10:58 a.m.	Insulin (4 units iletin, Lilly), subcutaneously.
11:55 a.m.	T. 38.93°; P. 220 to 240; R. 48. Anus relaxed. Blood sugar (12:00 m.) 0.047 per cent.
1:20 p.m.	T. 40.60°; P. 226; R. 48. Less somnolent than rabbit 823. Blood sugar (1:23 p.m.) 0.056 per cent.
2:35 p.m.	T. 40.71°; P. 210; R. 86 (shallow); animal wide awake. Blood sugar (2:38 p.m.) 0.065 per cent.
3:50 p.m.	T. 40.93°; P. 188; R. 84. Blood sugar (3:58), 0.085 per cent.
4:15 p.m.	After 12 minutes asphyxia, blood sugar 0.074 per cent.

The liver weighed 74.6 grams, and contained 3.52 per cent of glycogen.

In the next experiment, on a cat (829), the morphine and insulin were given practically simultaneously. In addition to the blood sugar the general symptoms and the rectal temperature were carefully studied, since morphine by itself produces so characteristic an effect upon the behavior of this animal, and also generally raises the body temperature markedly. It will be seen from the protocol that no trace of the typical morphine hyperglycemia, usually so pronounced in the cat, appeared at any time, notwithstanding the good store of glycogen in the liver. On the contrary, in an hour and a quarter after administration of the insulin and morphine the blood sugar had sunk from 0.13 to 0.046 per cent. Soon thereafter convulsions appeared, the blood sugar still falling, to 0.037 per cent, 2½ hours after insulin and morphine were given. The animal became comatose and an hour later, as it was seen that it would not recover spontaneously and was dying, it was killed to obtain

the liver for a glycogen estimation. The last blood sugar estimation gave only 0.033 per cent. Up to the time when the animal became comatose its behavior resembled that of a cat under morphine alone, but with a difference, the cat being less active and even in the spastic seizures, as it were, more languid. It lay down more than a cat simply under the influence of morphine generally does. Although the morphine symptoms were rapidly developed, as usual, it was evident that the insulin soon began to exert an influence upon these symptoms. As regards the body temperature, this sank steadily after the first hour, the characteristic morphine hyperthermia (5) being entirely absent. When the animal was killed the rectal temperature was nearly 3°C. below the initial temperature, although the animal occupied a cage near a warm radiator. The typical paradoxical reaction of the left iris (previously sensitized by removal of the corresponding superior cervical ganglion), persisted throughout the experiment. This, as we have abundantly demonstrated, is not dependent upon an increased output of epinephrin, although morphine does increase the rate of liberation of epinephrin in the cat (6), since it was equally well obtained in cat 828, whose epinephrin output had been suppressed by an appropriate operation on the adrenals. As regards the convulsions, they may also be seen, of course, after morphine alone, although from the degree of hypoglycemia reached it is probable that the insulin action predominated in eliciting them. But it is quite likely that their onset may have been favored by the morphine. It was not considered desirable to disturb the course of the blood sugar curve by administering glucose, which probably would enable us to discriminate morphine from insulin convulsions.

Condensed protocol. Cat 829. Female. Weight 2.65 kgm. The left superior cervical ganglion had been excised 47 days prior to the experiment.

9:40 a.m.	T. 38.78°; P. 178; R. 37. Blood sugar (9:42 a.m.) 0.130 per cent.
9:44 a.m.	50.0 mgm. morphine sulphate hypodermically.
9:45 a.m.	0.45 cc. iletin (Lilly, marked 50 units per cc.) hypodermically.
9:50 a.m.	Cat getting uneasy; defecated. Left pupil wider than right and left nictitating retracted.
10:00 a.m.	Cat getting active. Left pupil much wider than right and left nictitating retracted. Right nictitating still forward.
10:55 a.m.	T. 38.62°; P. 152 (forcible); R. 72. Cat makes jerky movements.
11:00 a.m.	Animal spastic. Blood sugar 0.046 per cent.
11:10 a.m.	Lying on side; jerky movements. At 11:20 a.m. convulsion for 20 seconds. Left pupil very wide and nictitating retracted. Right pupil narrower than left, and nictitating still forward.
12:00 m.	Convulsion, followed by semi-comatose condition.

12:18 p.m. T. 37.0°; P. 204; R. 52. Blood sugar (12:20 p.m.) 0.037 per cent. Opisthotonus. Comatose. Same at 12:35 p.m. It lies as if paralyzed. Cheyne-Stokes respiration.

1:15 p.m. T. 35.92°; P. 176; R. 28 (Cheyne-Stokes). Blood sugar 0.033 per cent.

1:25 p.m. Excised liver, which weighed 99.2 grams and contained 4.63 per cent of glycogen.

Since morphine increases the epinephrin output in cats, and insulin, as will be shown later, appears to have little, if any, effect upon the output, a similar experiment was made on a cat (828) whose epinephrin output had been suppressed by a preliminary operation. As will be seen from the protocol, the simultaneous administration of morphine and insulin to this animal produced effects quite similar to those observed in the normal cat 829. The sample of iletin used was the same in both experiments, which were done at the same time, in the same room. The general morphine symptoms were promptly developed, but were by and by overlaid by the insulin action, so that, as in the normal cat, the picture was no longer quite the same as in a cat under the influence of morphine alone. The state of excitation, which in the morphinized cat keeps the animal moving restlessly, with frequent spasms, seemed to be partially counteracted by the insulin, so that after a while the cat, either through weakness or apathy, gradually deepening into a comatose condition, lay down for long periods. This mixture, if it may so be termed, of the symptoms caused by both substances, was equally evident in the normal animal and in the animal which had undergone the adrenal operation. The rectal temperature, after a small rise, went on declining till the end of the experiment, as in cat 829, the morphine hyperthermia failing to develop. Whether the small preliminary rise of temperature was due to morphine or insulin cannot be known from this experiment. The administration of insulin by itself was not infrequently followed by some increase in rectal temperature, both in rabbits and cats, in the first part of the experiment; and later on the temperature might decline somewhat. An experiment on a cat, illustrating this, will be alluded to later. But the point we wish to emphasize here is that when morphine, a notable pyretic in the cat, was given in addition to insulin, a substance which can also cause a moderate increase in temperature, it was not a hyperthermia but a marked fall in temperature which was produced. The paradoxical pupil reaction (the left superior cervical ganglion having been extirpated prior to the experiment) was strongly marked throughout the experiment from a short time after administration of morphine. Although epinephrin could not have

been discharged from the adrenals, no essential difference in the behavior of the pupils and nictitating membranes was seen in this and in the normal cat.

Condensed protocol. Cat 828. Male. Weight 3.24 kgm. The right adrenal was extirpated, the left denervated and its medulla destroyed by curetting, 19 days prior to the experiment. The left superior cervical ganglion was excised 56 days before the experiment.

9:20 a.m. T. 38.14°; P. 160; R. 24. Blood sugar (9:25 a.m.) 0.103 per cent.

9:30 a.m. 50 mgm. morphine sulphate, and at 9:31 a.m., 0.5 cc. iletin (Lilly, marked 50 units per cc.) hypodermically.

9:40 a.m. Getting active; defecated; left pupil wider than right, left nictitating retracted. Same at 10:00 a.m.; salivated.

10:45 a.m. T. 38.80°; P. 112 (forcible); R. 45; cat getting jerky. Blood sugar (10:50 a.m.) 0.055 per cent. Cat quite spastic.

11:00 a.m. Lying on side and jerking. Left pupil very wide, wider than right, which is also dilated. Left nictitating retracted, right not.

12:05 p.m. T. 37.25°; P. 128; R. 48 (Cheyne-Stokes respiration beginning); comatose; opisthotonus. Blood sugar 0.045 per cent.

12:35 p.m. T. 36.81°; P. 96; Cheyne-Stokes respiration, gasping. Both pupils smaller than at 11:00 a.m., but left wider than right.

12:50 p.m. Blood sugar 0.040 per cent (blood almost black). Animal comatose, and looks as if paralyzed. Respiration stopped at 1:00 p.m. Liver immediately excised, weighed 104.6 grams and contained 3.34 per cent of glycogen.

It has been mentioned that insulin may cause some increase in the body temperature. An experiment on a normal cat (830) may be cited to illustrate this point. The specimen of iletin was the same as that used for cats 828 and 829. The maximum rise of temperature was not quite 1°C. above the initial temperature. This was maintained for several hours after the administration of the insulin, giving place to a fall about the time when the blood sugar content reached the minimum value of 0.038 per cent. The minimum temperature observed was about 1°C. below the initial value, and this coincided with a comatose condition of the animal, following several convulsions. The cat would undoubtedly have died, but was rescued by the subcutaneous injection of dextrose. The rapid and permanent recovery of this moribund animal was a very striking picture, as may be seen from the details in the protocol. As the animal recovered the temperature rose somewhat.

Protocol. Cat 830. Male. Weight 3.2 kgm.

9:50 a.m. T. 39.01°; P. 138; R. 32. Blood sugar (9:55 a.m.) 0.105 per cent.

9:57 a.m. 0.5 cc. iletin (Lilly, marked 50 units per cc.) subcutaneously.

10:38 a.m. T. 39.9°; P. 196; R. 32. Blood sugar (10:45 a.m.) 0.088 per cent.

11:00 a.m. Lying on side in cage, somewhat stupefied.

11:30 a.m. T. 39.84°; P. 134; R. 33. Condition about the same. Blood sugar (11:37 a.m.) 0.065 per cent.

1:03 p.m. Lying on side, more stupefied, but conscious. T. 39.85°; P. 108; R. 40. Blood sugar (1:06 p.m.) 0.052 per cent.

2:20 p.m. Slight convulsion for a few seconds. Respiration getting faster.

2:30 p.m. T. 38.74°; P. 66 (forcible); R. 120. Lying in semi-comatose state. Blood sugar (2:33 p.m.) 0.038 per cent. Indifferent to puncture by the needle.

2:35 to 2:50 p.m. 4 convulsions, each lasting a few seconds and followed by a a comatose condition.

3:06 p.m. T. 38.05°; P. 118 (forcible); R. 62. Comatose.

3:10 p.m. 3 grams dextrose hypodermically. Corneal reflex present.

3:30 p.m. Slightly less comatose; 1.5 gram dextrose injected.

3:39 p.m. Awake and less stupefied. T. 38.02°; P. 192; R. 42. Standing up, but still stupid. Blood sugar (3:40 p.m.) 0.066 per cent.

3:50 p.m. Begins to take interest in surroundings. A mouse, just caught, was placed in front of the cat, which at once seized and ate it. Drank water.

4:23 p.m. T. 38.37°; P. 204; R. 44. Cat is active; like a normal cat. Blood sugar (4:25 p.m.) 0.496 per cent. Cat returned to stock.

It has been stated earlier in the paper that insulin does not appear to exert any decided influence upon the rate of output of epinephrin. We made 3 experiments upon cats; in one with subcutaneous, in another with intravenous, and in the third with both subcutaneous and intravenous injection of insulin. As the results did not indicate that any marked or constant effect upon the output was caused, and as in experiments of this kind, with the methods of assay at our disposal, small apparent changes have no significance, it was not judged worth while to multiply the observations. The doses were nominally large, and the insulin samples were shown to be active in reducing the blood sugar in normal animals. The cats were necessarily fully anesthetized, ether being employed in two, and urethane in the third; and a marked hyperglycemia was present. Some reduction in the sugar percentage occurred in each case in the adrenal blood specimens collected at the longer intervals after the administration of the insulin. As the longest interval did not exceed $1\frac{1}{2}$ to 2 hours, and as the hyperglycemia might or might not have reached its maximum when the insulin was given, a very great or a very constant diminution in the blood sugar could probably not be expected. Still, in one of the animals (cat 841) it was reduced about 50 per cent, $1\frac{1}{2}$ hours after insulin. In the earlier specimens, collected only a few minutes after giving insulin, there was, of course, no reduction in the blood sugar.

In cat 840, a male, weighing 3.46 kgm., anesthesia was begun at 8:50 a.m. A specimen of adrenal blood (5.4 grams in 2 minutes) was collected at 9:32 a.m. It contained 0.340 per cent of sugar. At 9:45 a.m., 2 cc. of iletin (Lilly, marked 5 units per cc.) was injected subcutaneously, and 15 minutes thereafter (10:00 a.m.) a specimen of adrenal blood (5.85 grams in 2 minutes) was collected. It contained 0.364 per cent of sugar. At 10:34 a.m. (49 minutes after insulin) a third specimen of adrenal blood (4.0 grams in 2 minutes) was obtained. It had 0.328 per cent of sugar. At 11:47 a.m. (2 hours after insulin) adrenal blood was again drawn (3.55 grams in 2 minutes) with 0.267 per cent of sugar. Blood taken from the abdominal aorta a few minutes later had a sugar percentage of 0.245. The blood flow was good during collection of each of the specimens, and the assay showed that the epinephrin concentrations in them all varied approximately inversely as the blood flow, i.e., the rate of output was not sensibly altered by the insulin. It was reckoned at about 0.0002 mgm. epinephrin per kilogram of body weight per minute in the last specimen, near the ordinary average and quite within the normal range in etherized cats.

In cat 836, a male, weighing 2.97 kgm., urethane (5 grams) was injected subcutaneously at 8:50 a.m. A little ether was given when cutting the skin during the operative procedures, and only once thereafter. At 10:03 a.m. a specimen of adrenal blood was obtained. Then at 10:10 a.m., 0.5 cc. of iletin (Lilly, marked 40 units per cc.) was injected intravenously. Nine minutes later an adrenal blood sample was drawn. At 10:41 a.m. (21 minutes after injection of insulin) a third adrenal blood specimen was collected. At 11:34 a.m. (84 minutes after insulin) another specimen was drawn, and then a sample from the abdominal aorta. The maximum blood sugar content was found in the second adrenal specimen (0.452 per cent); the fourth specimen contained 0.366 per cent, and the blood from the aorta at the end of the experiment 0.356 per cent. The epinephrin assay gave 0.0003 mgm. per kilogram per minute as the output for the adrenal sample drawn 9 minutes after insulin, and the same for the sample taken 21 minutes after insulin, an amount very little above the average in urethanized cats. But as the output for the specimen taken before injection of insulin was only about half as great (being considerably below the normal average but still quite within the normal range), an apparent small increase followed the administration of insulin. During collection of the last adrenal specimen the flow was small, the blood pressure having fallen, and the epinephrin concentration (1:800,000) was too near the maximum limit to permit the conclusion that the calculated output (0.00018 mgm. per kgm.) is not below the real output at this time.

In the next experiment (cat 841), with a much larger total dose of the same samples of insulin, injected both subcutaneously and intravenously, no increase in the output was found. On the contrary, a marked transient decrease was seen in the adrenal blood specimen obtained immediately after injection of insulin. There seems to be no doubt that there was a genuine decrease in the output at this time. For the concentration of epinephrin assayed in the specimen was very much less than in the initial, or any of the other specimens, and this was not accounted for by the difference in the rate of blood flow. On the theory that epinephrin and the pancreatic internal secretion act as antagonists in the regulation of the carbohydrate metabolism and the blood sugar content (for which, as already remarked, there is no experimental basis), it might seem advantageous that the pancreatic active substance should have the power of inhibiting the output of epinephrin. It would be simply fantastic to read any such meaning into our observation, even if it were constantly obtained. For even if we were dealing with a pure preparation of the pancreatic hormone, it could not be known at present whether the dose employed did not vastly exceed anything normally given off by the pancreas. But since there were certainly other substances present in these pancreatic extracts, including substances added to preserve them, one or more of which might affect the epinephrin output, the question need not be discussed further. All we desired to do was to supplement our other observations on insulin by testing whether the extract, such as it was, did or did not markedly affect the output.

The protocol of the experiment on cat 841 follows. As it was thought possible that with a reduction of the glycogen store insulin might exert a secondary action on the epinephrin output, associated with the diminution in the blood sugar, this animal was deprived of food for some time before the experiment. Since the animals were all anesthetized, and already had a hyperglycemia before administration of insulin, it has not been possible, of course, to observe the output during insulin convulsions, to see whether the epinephrin-secretory nervous mechanism was also stimulated.

Protocol. Cat 841. Female. Weight 2.375 kgm. Only water for 4 days before the experiment, with the exception of a little milk on the second day of the fasting period.

8:40 a.m. Ether begun.

9:27 to 9:30 a.m. Adrenal blood (4.2 grams in 3 minutes), sugar content 0.27 per cent.

9:37 a.m. 1 cc. iletin (Lilly, marked 40 units per cc.) subcutaneously. Same sample as used in cat 836.

9:42 to 9:44 a.m. Slow intravenous infusion of 2.5 cc. of another sample of iletin (Lilly, marked 5 units per cc.) Same as used in cat 840.

9:45½ to 9:47½ a.m. Adrenal blood (4.1 grams in 2 minutes); sugar content 0.286 per cent.

10:24 to 10:27 a.m. Adrenal blood (3.3 grams in 3 minutes), sugar content 0.222 per cent.

11:04 to 11:10 a.m. Adrenal blood (2.7 grams in 6 minutes). Blood from the abdominal aorta was now collected. It contained 0.143 per cent of sugar. Left adrenal weighed 0.150 gram, right 0.151 gram. Sugar estimations by Folin-Wu method. Also by Shaffer-Hartmann method, with good agreement.

The initial adrenal blood specimen, before insulin, contained about 1:2,000,000 epinephrin, giving an output of 0.0003 mgm. per kilogram. The specimen taken immediately after insulin contained not more than 1:15,000,000 epinephrin, corresponding to an output of 0.00005 mgm. per kilogram per minute, or only about one-sixth of the initial value. The specimen collected 40 minutes after insulin had a concentration of about 1:1,800,000, giving an output of 0.00025 mgm. per kilogram per minute, practically the same as the initial value. The last adrenal specimen, taken 80 minutes after administration of insulin, had a concentration of 1:700,000 epinephrin, corresponding to an output of 0.00027 mgm. per kilogram per minute. The blood flow, however, was small during collection of this specimen, and the concentration about the maximum. As in all the experiments, the samples collected after insulin was given were assayed with indifferent blood taken after the insulin injection. This produced no change in the rabbit intestine segment when it was made to replace indifferent blood collected before injection of insulin. Ringer's solution containing a very high concentration of iletin caused inhibition of the segment. Whether this was due to the preservative or to substances in the pancreatic extract, was not ascertained.

SUMMARY

Morphine hyperglycemia, which has been shown to depend in some way upon the integrity of the adrenals, is counteracted by insulin, just like other experimental hyperglycemias (piqûre, ether, asphyxia) in which the adrenals are not essentially concerned. As great a reduction of blood sugar was observed in a normal cat as in a cat whose adrenals had been interfered with by a preliminary operation to suppress the epinephrin output, when insulin and morphine were administered to

both in the same doses, although in the normal cat the morphine action in raising the blood sugar content would be expected to be decidedly greater than in the cat which had undergone the adrenal operation.

The general symptoms were the same in the normal and the previously operated animals, a kind of combination of the symptoms caused by morphine alone with those caused by insulin alone being observed. The course of the body temperature was also the same. The characteristic hyperthermia caused by morphine in cats was not developed when insulin was also given.

In three cats, in which the influence of insulin upon the epinephrin output was investigated, no definite effect of any moment could be made out.

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THE ACTION OF INSULIN ON ADRENALECTOMIZED RABBITS[1]

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The existence of a close relationship between the adrenals, especially the medulla, and the pancreas in the regulation of the carbohydrate metabolism has been maintained by a number of writers. In examining the relation of the adrenals to pancreatic diabetes in dogs (1) and the effect (especially in cats) of insulin upon morphine hyperglycemia (2), a form of experimental hyperglycemia which, unlike the other forms investigated, appears to depend closely upon the integrity of the adrenal bodies (3), we have already alluded to this question. No evidence was obtained which could be interpreted in favor of current theories linking the adrenals or the adrenal medulla in any specific way to the internal secretory mechanism of the pancreas. It seemed still of interest, as bearing upon the same general question, but also because of the use of rabbits in standardizing insulin(4), to see whether the action of insulin or rabbits which had survived total adrenalectomy differed noticeably from its action on normal animals. No difference was made out which could be attributed to absence of the adrenals. The following protocols illustrate the results on an adrenalectomized animal (842) and a normal control animal (843). The rabbits were in adjacent cages in the same room during the experiment. The iletin specimen was the same for both. It was injected subcutaneously.

Protocol. Rabbit 842. Female. Weight 1.82 kgm. Right adrenal excised 69 days and left adrenal 50 days prior to the experiment.

9:25 a.m. T. 38.28°; P. and R. too fast to count. Blood sugar 0.114 per cent.
9:30 a.m. 1 cc. iletin (Lilly, marked 5 units per cc.)
10:20 a.m. T. 38.66°; P. and R. too fast to count. Blood sugar 0.067 per cent.
11:15 a.m. T. 38.48°; Blood sugar 0.048 per cent.
12:15 p.m. T. 38.78°; P. 260 +; R. 165. Blood sugar 0.042 per cent.

[1] A note was published in the Proc. Soc. Exper. Biol. and Med., 1923, xx, 339.

2:10 p.m. T. 39.03°. Blood sugar (2:20 p.m.) 0.071 per cent.

2:25 p.m. Animal weak, restless, with symptoms such as frequently precede convulsions; injected 1 gram dextrose hypodermically.

3:00 p.m. T. 39.70°. Blood sugar 0.095 per cent. The animal recovered completely.

Protocol Rabbit 843. Normal male. Weight 1.80 kgm.

9:45 a.m. Blood sugar 0.10 per cent. T. 39.2°; P. 216; R. 40.

9:53 a.m. 1 cc. iletin (Lilly, marked 5 units per cc.)

10:34 a.m. T. 38.9°; P. 216; R. 96. Blood sugar 0.047 per cent.

11:05 a.m. A convulsion. Blood sugar (11:10 a.m.) 0.052 per cent. T. 39.05°; P. too fast to count; R. 76.

12:00 m. Convulsion, during which death occurred. Blood at once obtained from heart, contained 0.077 per cent sugar.

It will be seen that the blood sugar diminished more rapidly in the control than in the adrenalectomized rabbit, although as low a level was eventually reached in the latter (0.042 per cent in rabbit 842, and 0.047 per cent in rabbit 843). If the physiologically liberated epinephrin was really holding up the blood sugar content against the antagonistic action of the pancreatic hormone, the blood sugar ought to fall more rapidly in the adrenalectomized animal. Of course such a difference as was seen in these two rabbits cannot be used as evidence in this matter. For it is well known that there are great variations in the response of different rabbits to the same dose of insulin. It is no doubt a matter of accident, possibly depending partly upon the glycogen store, that the adrenalectomized rabbit had not developed convulsions in 5 hours after the insulin was given, whereas the control animal had a convulsion in little over one hour, and died in convulsions in 2 hours. Whether rabbit 842 would eventually have shown convulsions is unknown, as it was restored by an injection of dextrose. In another adrenalectomized rabbit (835) convulsions began in less than $1\frac{1}{2}$ hours after administration of insulin when the blood sugar had sunk to 0.039 per cent and continued at intervals till the animal was rescued by injection of dextrose. We understand the unit of iletin as given in this and our other two papers (1), (2) to represent one-fifth of the unit originally used by Macleod. The nominal dose was greater than in rabbits 842 and 843, but our specimens were kept a longer or shorter time in the ice chest before being used. In any case it is doubtful what value should be attached to nominal differences in the dosage in such work. The main point is that all the samples used were quite active in the doses employed.

Protocol. Rabbit 835. Weight 2.27 kgm. Right adrenal excised 60 days, and left adrenal 28 days prior to the experiment.

9:00 a.m. Blood sugar 0.111 per cent.
9:03 a.m. 0.5 cc. iletin (Lilly, marked 40 units per cc.) subcutaneously. T. 38.55°; P. 260; R. 140.
10:00 a.m. T. 39.16°; P. 280 to 300; R. 42. Blood sugar 0.059 per cent.
10:30 a.m. Convulsion. Blood sugar (10:35 a.m.) 0.039 per cent.
10:48 a.m. Convulsion. Blood sugar (10:52 a.m.) 0.045 per cent.
11:00 a.m. Injected 0.6 gram dextrose subcutaneously. Rabbit recovered in 10 to 15 minutes.
11:45 a.m. Blood sugar 0.081 per cent.
1:55 p.m. T. 40.45°; P. 300 +; R. 120. Blood sugar 0.106 per cent.
3:25 p.m. T. 40.68°; P. too rapid to count. R. 112. Blood sugar 0.113 per cent.
4:00 p.m. Animal returned to cage, quite recovered.

In the last experiment to be cited (rabbit 822) the adrenals had been removed many months previously. No convulsions developed, although the blood sugar sank to 0.048 per cent in $1\frac{1}{2}$ hours. From this point the recovery of the sugar content was comparatively rapid, a normal level, 0.1 per cent and upwards, being present $3\frac{1}{2}$ hours after injection of insulin. Even $2\frac{1}{4}$ hours later ($5\frac{3}{4}$ hours after insulin was given), when the blood sugar content was actually at the initial level, and had been normal for 2 to 3 hours, the clinical condition of the animal did not seem to have improved. It remained lying on its belly, stupefied but not unconscious. In this case then it would seem that the condition of the animal was due to something else than the substance in this preparation of insulin responsible for the reduction in the blood sugar. That the action of the substance which causes diminution in the sugar was long since over is rendered probable, not only by the return of the sugar percentage to normal but by the fact that a period of asphyxia sent the percentage up to 0.267, just as if insulin had never been given. The usually brilliant results of the administration of dextrose in animals dying after insulin would seem to indicate that in many or most of the specimens the substance responsible for the continued depression in rabbit 822 is not present.

Protocol. Rabbit 822. Male. Weight 1.53 kgm. The animal was 11 months old. The right adrenal was excised $8\frac{1}{2}$ months and the left adrenal $7\frac{1}{2}$ months before the insulin experiment.

9:50 a.m. Blood sugar 0.110 per cent.
9:55 a.m. 10 units iletin (Lilly) given subcutaneously.
10:35 a.m. Blood sugar 0.059 per cent.
11:30 a.m. T. 38.0°. Blood sugar 0.048 per cent.
12:20 p.m. Rabbit lying on its belly, stupefied but not unconscious.
12:30 p.m. Condition unchanged. Blood sugar 0.072 per cent.

1:30 p.m. Condition the same. Blood sugar 0.099 per cent.
2:30 p.m. Condition unchanged. Blood sugar 0.103 per cent.
3:45 p.m. Condition the same. Blood sugar 0.111 per cent.
4:10 p.m. After asphyxia for 15 minutes (off and on) blood sugar 0.267 per cent. Animal dying while the blood specimen was being collected.

Urine squeezed out of the bladder just before asphyxial period gave no reduction with Fehling. At necropsy no accessory adrenals were found macroscopically. The liver weighed 70 grams and contained 3.5 per cent of glycogen.

SUMMARY

The action of insulin on rabbits which have survived complete removal of the adrenals does not differ noticeably from its action on normal rabbits.

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Note added June 20, 1923. Since the above was written we have been informed that the specimen of iletin used in the last experiment (rabbit 822) was "the last lot of crude material made by the original method." It was marked "distributed Dec. 8, 1922." Since that time "only more highly purified material has been shipped for human cases." We do not know whether the preparation would have given similar results in other animals, as in the other experiments in which we used it morphine was also administered.

NEW EXPERIMENTS ON THE NATURE OF THE SENSATION OF THIRST

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The theory that thirst is of the nature of a general sensation, with a secondary local reference to the pharynx, has in the past received widespread and almost universal credence. This view maintains that the loss of the water content of the tissues (e.g., in diarrhea, polyuria, water deprivation, etc.) increases the salt concentration of the body fluids, which condition is responsible for the origin of afferent impulses from the various viscera, or for the direct stimulation by the hypertonic blood of the unknown center, in which the sensation of thirst is represented.

Thirst has also been considered as essentially a local sensation limited to the mucous membrane of the mouth and throat; a theory, the chief exponents of which have been investigators, who abolished thirst by the application of cocaine to the mucous membrane of the mouth and throat in thirsty dogs and in patients afflicted with diabetes insipidus.

Cannon, in his classical and admirable article on the physiological basis of thirst, has submitted a new explanation, which is in agreement with all experimental data to date. According to Cannon (1), thirst is due to a relative drying of the mucosa of the mouth and pharynx. This drying is due to either a diminution or absence of the salivary secretion, a condition brought about by any of the dehydrating factors mentioned above under the general causes of thirst, or by such local factors as mouth-breathing, prolonged singing or speaking, etc. In his own words: "The diminishing activity of the salivary glands becomes a delicate indicator of the bodily demand for fluid." The thirst produced by physiological doses of atropine, by such emotional states as anxiety and fright, by ligation of the salivary ducts (Bidder and Schmidt), he explains as due to a diminution or absence of the salivary secretion; while the alleviation of thirst by chewing insoluble substances such as

paraffin, or by taking into the mouth fruit juices such as lemon juice, may be attributed to the mechanical and chemical stimulation, respectively, of the mucous membrane, with a resultant increase in the salivary secretion. At this point it might well be mentioned that these two latter measures are very well known by the laity. It is a quite common practice for long distance runners to suck a lemon at intervals. Soldiers on long marches in arid regions appreciate the antidipsic properties of a button or pebble on the tongue.

If an increase in the flow of salivary secretion will appease the desire to drink, then it might be inferred that pilocarpine would be an efficient agent in relieving thirst. With this idea in mind, the following experiments were performed.

Methods and results. Rabbits were used as experimental animals. They were subjected to seven-day periods of fasting, food and water both being withheld. At the end of this period, pilocarpine hydrochloride was administered subcutaneously, in doses of 0.5 cc. of 1 per cent, per kilogram of body weight. When salivation became profuse, measured amounts of water were placed within their cages and left for one hour. The control animals were given a hypodermic injection of equivalent amounts of water, so as to eliminate the psychic factor of fear, their access to water being limited to one-half hour.

It could be imagined that after one week of deprivation thirst would be very intense. Although the pangs of hunger diminish after the third day of fasting, thirst continues and becomes even more pronounced. Underhill and Roth (2) have demonstrated that complete deprivation of water for one week leads to blood concentration in rabbits. It is to be inferred that a diminution in salivary secretion is concomitant with this condition. These identical blood changes have been similarly induced in dogs by Underhill and Kapsinow (3). In the animals of this latter experiment thirst was so intense that when water was offered them, they immediately drank from 350 to 500 cc. This water introduction not only satisfied the thirst but diminished the hemoglobin concentration from 125 per cent to 100 per cent or normal.

The rabbit controls in this experiment drank from 62 to 137 cc. within the first half-hour. The rabbits, salivating from pilocarpine, either refused to drink or, as in two cases, drank 15 and 25 cc. of water, within the hour. This difference can be interpreted as due to quenching of their thirst by the drinking of their own body fluids. Now the sialogogue action of pilocarpine is due to the stimulation of the myoneural junction of the parasympathetic (cranio-sacral autonomic)

nerves, hence affecting the secretory fibers of the salivary glands, especially of the glossopharyngeal and chorda tympani nerves. Although pilocarpine alleviates thirst, it does so because of the continuous local relief afforded the mucosa by the saliva. This fact tends to disprove the theory that thirst is of the nature of a general sensation, because pilocarpine, instead of causing a dilution of the blood and tissue fluids, conversely brings about an exceptionally high blood concentration, especially when its administration is superimposed upon prolonged water deprivation (2).

TABLE 1

RABBITS	WEIGHT		FLUID INTAKE	
	Before fasting	After fasting		
	kgm.	*kgm.*	*cc.*	
Control 1	2.30	1.80	62	Length of period in which control rabbits had access to water—one-half hour
Control 2	2.40	1.90	74	
Control 3	2.30	1.84	137	
Control 4	2.70	1.90	118	
Control 5	2.03	1.64	102	
Control 6	1.70	1.16	112	
Experiment 1	2.60	2.00	0	Rabbits received 0.5 cc. per kgm. of body weight, of 1 per cent pilocarpine hydrochloride hypodermatically. Access to water for one hour
Experiment 2	2.50	1.80	0	
Experiment 3	2.70	2.04	25	
Experiment 4	2.60	2.10	15	
Experiment 5	1.50	1.20	0	
Experiment 6	2.00	1.60	0	
Experiment 7	2.70	2.00	0	
Experiment 8	1.65	1.30	0	
Experiment 9	1.90	1.55	0	
Experiment 10	2.30	1.90	7	
Experiment 11	2.00	1.70	0	
Experiment 12	1.85	1.40	6	

While this investigation was in progress, attention was called to the instances cited by Weir, Larson and Rowntree (4), where pilocarpine failed to relieve the thirst in several cases of diabetes insipidus. The local application of cocaine to the mucosa was also unsuccessful. The cause of thirst in this peculiar affliction has never been determined. A number of clinicians have reported that thirst and polydipsia precede the polyuria. All of these facts taken into consideration have influenced the above quoted authors in assuming that thirst in diabetes insipidus is more than the mere expression of dryness of the oral mucous membrane.

CONCLUSION

Cannon's theory of thirst is substantiated by the fact that pilocarpine, administered to rabbits deprived of water for seven days, relieves thirst by stimulating the flow of saliva.

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FUNCTIONS OF THE CERVICAL SYMPATHETIC AS MANIFESTED BY ITS ACTION CURRENTS

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Since the discoveries of Claude Bernard the cervical sympathetic has been the subject of much investigation. The action currents of this nerve, which to our knowledge have not hitherto been recorded, throw much light on the manner in which it functions in the almost intact body. It might be argued that the functions of the nerve are sufficiently apparent from the phenomena exhibited by the organs innervated by it, viz., the salivary glands, certain ocular mechanisms, and some blood vessels of the head. But as each of these has at least two innervations it is difficult to determine in any given case which is the one in action. Compare the reaction of the pupil to nociceptive stimulation (pathic reflex). Thus with the eyes fixed on a distant object and exposed to a constant illumination, a pin-prick anywhere on the skin causes sudden dilatation of the pupils. Compare Bernard (1) who showed that stimulation of any sensory nerve evoked this reflex dilatation. The brain plays a prominent part in the mechanism here involved. A detailed and clever description of a related phenomenon is given by Stärcke (2) who points out that there will always be a dilatation of the pupil when our attention to outward objects gives place to an inward sensation (feeling). But through what pathways do the stimulus effects reach the iris? It cannot be established a priori that it is via the sympathetic as the iris is also under oculomotor influence, inhibition of which, as well as sympathetic excitation, induces dilatation. Registration of the neural action currents, it was thought, might yield information on this point.

METHOD. Our procedure was mainly that adopted in a previous inquiry into the action-currents of the vagus (3) and depressor (4)

[1] This research was conducted with the continuous collaboration of the laboratory assistants, all of whom we wish to thank and in particular Doctor Bijtel and Mr. Hoogerwerf.

nerves. A rather long portion of the cervical sympathetic was prepared and severed toward the distal (cephalic) end. The cut surface of the preparation was brought into contact with a Porter non-polarizable boot. At about a distance of 1 cm. from this the nerve trunk was made to rest on a second electrode and then suspended from a pair of glass hooks. Between the points of contact with the hooks the nerve bulged downward. With the electrodes attached to a sensitive string galvanometer, neither the spontaneous movements of the animal, nor the oscillations imparted intentionally, by means of an ebonite pincette, to the fragment of the nerve that hung down between the animal and the first (caudal) glass hook, affected the position of the string. The galvanometer, the animal and the apparatus for measuring the blood pressure were all insulated so as to make the total insulation-resistance amount to from 10^{-9} to 10^{-10} ohms. Perfect insulation must be maintained when the animal is connected with the apparatus for artificial respiration. To this end a glass cannula, kept dry by heat during the experiment, was interposed between the trachea and the tubes of the apparatus. Without this precaution the inner walls of the tubes became moist and the insulation was broken. The heating was effected in a simple and practical way by passing an electric current through a wire wound round the cannula. There was only one earth connection, viz., at the thigh under the skin. When the severed sciatic nerve was to be stimulated, earthing was effected from the trunk of the nerve itself at a spot between the animal and the free, severed nerve-end where the stimulating electrodes were attached.

The blood pressure was registered in a very simple way. As it was merely necessary to ascertain whether or not the blood pressure responded to the stimuli applied, only an ordinary mercury-manometer was used. The movements of the mercury were transmitted by an air tube to a recording tambour which was arranged in front of the slit of the camera plate.

If an electric current be employed as stimulus the exact moment of its application must be recorded. To this end a second string-galvanometer was ranged beside the first in such a way that the two strings were represented simultaneously on the photographic plate. The string that registered the moment when the stimulating current was thrown in was connected with a coil in which the stimulating current induced a current of just sufficient strength to give the string a suitable deflection. The moment the stimulation was started was thus indicated with absolute precision.

As sound stimulus a motorist's claxon was used. The sound waves from this instrument were caught up by a telephone transmitter placed near the head of the animal. The telephone current was conducted through the second (signal) galvanometer which recorded the moment the sound waves reached the animal's ear.

When using heat as the stimulus we were unable to mark the moment of stimulation precisely. A stout copper bolt was fitted at its upper end with an insulated handle, the lower end being cone-shaped. To the apex of the cone a small silver cylinder was attached. The bolt was heated in a water bath up to 70°C. after which its temperature remained rather constant for a considerable time. Held by the insulated handle the silver cylinder was pressed at some spot against the body of the animal for a given time. At the same time an assistant recorded a signal on the photographic plate. Sometimes the action currents were derived from both cervical sympathetic nerves simultaneously. Then a third string-galvanometer optically arranged in series with the first was brought into operation (5).

We are aware that the method adopted by us has its shortcomings. Aside from some deficiencies of minor importance the curves display a main fault. They only approximately indicate the precise fluctuations of potential developing in the functioning nerve. When registering a human electro-cardiogram by means of a suitable string-galvanometer a curve may be obtained which practically gives, in every detail, a true picture of the varying electromotive forces under examination. But in order to record our electro-sympatheticograms we have had to increase considerably the sensitivity of the galvanometer. This involved a slowing of the deflection of the string. The neural action-currents though represented by oscillations whose rate exceeds that of the string movement can nevertheless be visibly registered in the curves although the amount of the string deflection is not a precise index of the amount of the potential difference that has developed. To make up for this deficiency two courses were open to us: *a*, We might correct the curves, *i.e.*, we might construct new ones, using the data deducible from the properties of the string and the shape of the registered curves (6). But this is a tedious, time-consuming operation. *b*, We might avail ourselves of thermionic valves by which the nerve currents are amplified. With these sufficiently amplified to impart an adequate deflection to an insensitive, fast deflecting string, the curves recorded give a true picture of the oscillations of potential. This method, which is preferable to the first one, had not been fully prepared when our experiments were made. However, we feel confident that before long we shall be able to apply it.

THE REGISTERED CURVES. Only a few of the registered curves will be here reproduced. They are all taken from curarized animals under artificial respiration. This latter had to be carefully regulated so as to avoid over-aëration which it was found enfeebled or altogether abolished sympathetic function (acapneic shock). To economize space the copies of the broad photographic plates have been cut into two or more pieces along the abscissae and pushed up in the direction of the ordinates so that the space occupied by the reproductions has been considerably diminished. This does not interfere with the accuracy of the time-measurements, notably of the latent periods, since the ordinates correspond exactly. The fact that every 5th ordinate is slightly thicker than the others does away with the difficulty that might otherwise arise in adjusting the strips of paper. In the figures *S* signifies signal, *E*, electrosympathicogram, and *B*, blood pressure. A scale division along the abscissae equals 0.1 second in all the figures.

Figure 1 is a simple instance of an electrosympathicogram, *E*, from a cat. The current is derived from the right cervical sympathetic. On *S* is indicated the moment of stimulation of the left sciatic nerve. A scale-division along an ordinate corresponds to a potential difference of 4×10^{-6} volts. A scale division along an abscissa represents 0.1 second as in all the figures.

It will be noted that even when apparently quiescent the electrosympathicogram is not a straight line but exhibits slight fluctuations which, however, are considerably enlarged upon sciatic stimulation. As may be seen from the photo the latent period of the stimulation amounts to 0.5 mm. corresponding to 0.05 second. The blood pressure responded to the stimulus by rising from 90 to 161 mm. Hg. The left pupil became considerably dilated.

Figure 2 represents the electric reaction of the cervical sympathetic for sound stimulation administered by a claxon in the manner previously described. The latency amounts to about 0.16 second. The blood pressure was low, viz., 60 mm. Hg and was not altered by the stimulus. The aspect of the electrosympathicogram differs from that in the one resulting from sciatic stimulation. This is presumably due to the short duration of the claxon-sound which, as marked by the signal, was about 1 second. The electric reaction of the cervical sympathetic was of still shorter duration, viz., about 0.4 second. One ordinate scale-division equals 7×10^{-6} volts.

Thermal stimulus (70°C.) applied with bolt previously described. The right cervical sympathetic rested upon the electrodes and the

sensitivity of the string had been regulated so that 1 scale-division along the ordinates corresponded to 5.5×10^{-6} volts. When the stimulus was pressed against the skin of the right side of the lower abdomen the change in potential in the right sympathetic, as represented in the registration curve, was less, by 13.5 microvolts, than when the stimulus was applied to the left (heterolateral) side of the abdomen. The blood pressure rose from 75 to 78 mm. Hg. This rise, which could be read from the mercury manometer, was too slight for registration in the curve of fig. 3.

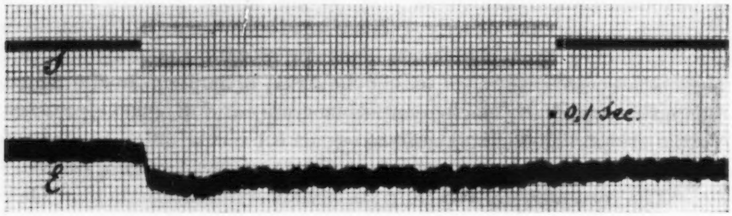

Fig. 1. Cat. Stimulation of left sciatic; derivation from right cervical sympathetic; 1 scale-division of an ordinate equals 4 microvolts.

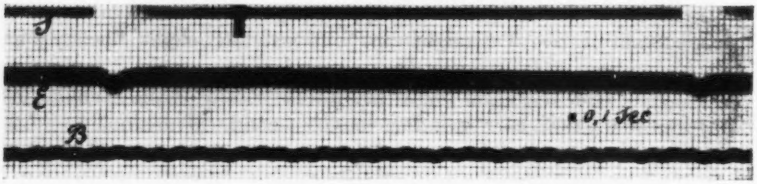

Fig. 2. Cat. Sound stimulus; derivation from the right cervical sympathetic; 1 scale-division of an ordinate equals 7 microvolts.

We have made only a few experiments with dogs.

The right vagosympathetic rested upon the electrodes and, in accordance with previous results (3), it elicited electric oscillations in which two rhythms are distinguishable. The large, slow fluctuations correspond to the movements of the artificial inflation of the lungs, while the small, frequent waves coincide with the heart rhythm. Owing to the slowness of the string the former are reproduced more

distinctly than the latter, which are barely visible. Occasionally, however, the curves exhibit small fluctuations which correspond to the waves of the blood-pressure curve, *B*. Some of these have been designated by a cross. The signal records the onset of left sciatic stimulation. A distinct reaction in the electrovagosympathicogram and a rise of the blood pressure (from 85 to 125 mm. Hg) can be noted in fig. 4.

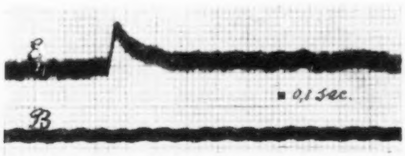

Fig. 3. Cat. Heat-stimulation with bolt applied to the skin of the left side of lower abdomen; derivation from the right cervical sympathetic; 1 scale-division of an ordinate equals 5.5 microvolts.

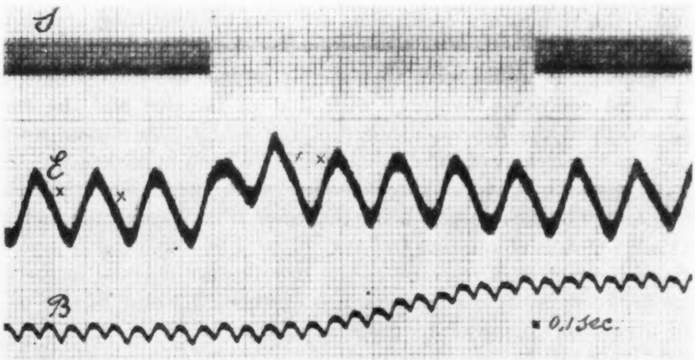

Fig. 4. Dog. Stimulation of the left sciatic; derivation from the right vagosympathetic; 1 scale-division of an ordinate equals 1.7 microvolt.

Owing to the large respiration waves in the curves the latency cannot well be measured. It may be as long as in the cat, viz., from 0.04 to 0.06 second. The rise in blood pressure begins much later, about 1.5 to 1.7 second after the commencement of the stimulation. A scale-division of an ordinate equals 1.7×10^{-6} volts.

In the cat also some curves have been registered from the vagosympathetic trunk. This method simplifies the operation, since it is not necessary to separate the vagus from the cervical sympathetic which, in the cat as in the dog, are sometimes closely bound together by connective tissue. The method has the further advantages of affording opportunity for immediate comparison of the electric fluctuations in the sympathetic with those in the vagus. In a way this is a test of the validity and the accuracy of the experiment. However, it has also its drawback as the rhythmic oscillations in the curves interfere with exact measurement of the latent period.

In figures 5 and 6 bilateral derivations are registered Three galvanometers are in operation simultaneously: one for the sig nal, *S*, marking the onset and duration of the stimulation; a second, which takes the right vagosympatheticogram *Er*, and a third which registers *El*, the left vagosympatheticogram. The simultaneous registration of the nerve-currents on both sides renders a comparison between the homolateral and the heterolateral effects easier and more reliable.

In figure 5 artificial respiration is employed in the ordinary way, while in figure 6 it has been momentarily stopped. Accordingly in figure 5 the respiratory oscillations are seen whereas they are absent in figure 6. There is a striking prominence of the heart rhythm in the neurograms. It would seem that in this respect the electrovagogram of the cat compares favorably with that of the dog and the curve obtained from the depressor nerve of the rabbit. As far as we are aware, no vagograms of the cat have hitherto been published.

In figure 5 the right, in figure 6 the left, sciatic nerve was stimulated, the intensity of the stimulus being the same in both cases, the distance between the coils amounting to 5 cm. It will be noted that the elevations of the curves in response to the stimulus are unequal in the two figures. In figure 5, *Er* shows a rise of about 3.5 and *El* one of 13 scale-divisions. In order to properly appreciate these elevations we should consider that the sensitivity of the galvanometers was not the same in the four cases. Thus for the two neurograms of figure 5, and for *Er* of figure 6, a scale-division of an ordinate is equivalent to 5×10^{-6} volts. The sensitivity of the galvanometer was made 2.5 times greater for the other curve, i.e., for *El* of figure 6, so that here one scale-division of an ordinate is 2×10^{-6} volts. When expressing the rise of the curve in volts we find in figure 5 upon right sciatic stimulation the values to be: for *Er*, 10, and for *El*, 50 microvolts; in figure 6, upon left sciatic stimulation the values are: for *Er*, 16.5, for *El*, 26 microvolts.

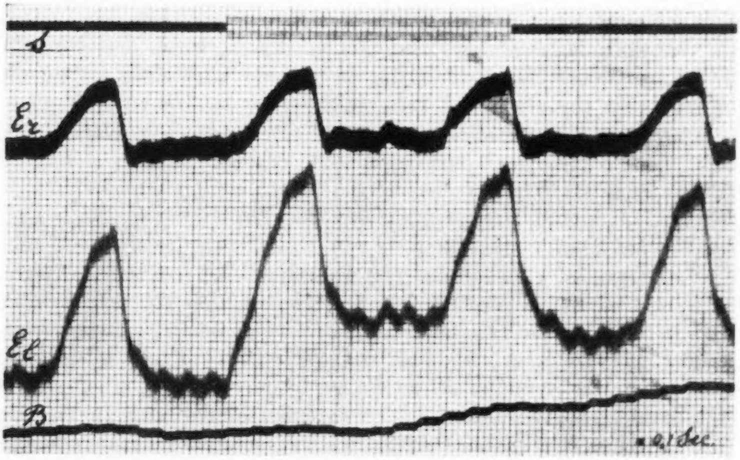

Fig. 5. Cat. Stimulation of right sciatic; bilateral derivation from the vagosympathetic. *Er*, right, and *El*, left, electroneurogram. In either 1 scale-division of an ordinate equals 5 microvolts.

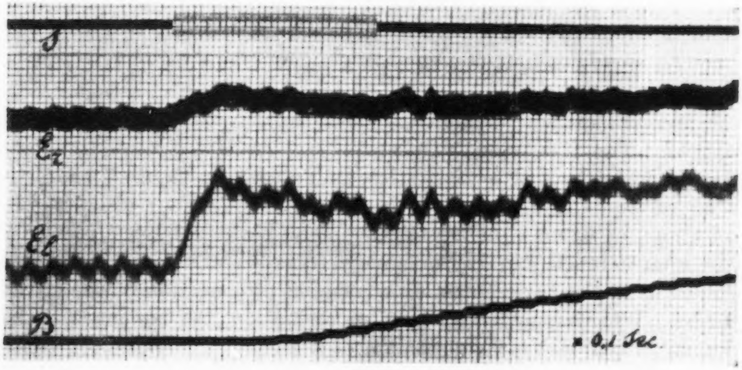

Fig. 6. Same cat as in figure 5. Artificial respiration stopped; stimulation of left sciatic; bilateral derivation from the vagosympathetic. *Er*, right electroneurogram, in which 1 scale-division of an ordinate equals 5 microvolts; *El*, left electroneurogram, in which 1 scale-division of an ordinate equals 2 microvolts.

From these values one would be inclined to conclude that the left and the right cervical sympathetic do not react with the same intensity for the same stimulus. But this conclusion, which may be right in this particular case, cannot be of general value since we cannot be sure that the electrodes were equivalently applied to both vagosympathetics. Moreover, adventitious factors might invalidate minute comparison. However, comparison on another basis yields more reliable results. When comparing the effects of right and left sciatic stimulation on the same sympathetic nerve, as done in the animal from which figure 3 was taken, it was evident that heterolateral produced greater effects than homolateral stimulation. Compare Byrne (7). In that animal upon right sciatic stimulation the deflection in *Er* was 10 microvolts as against 16.5 microvolts upon left sciatic stimulation. On the other hand the deflection in *El* upon right sciatic stimulation was 50 microvolts as against 28 microvolts upon left sciatic stimulation. So the effect of heterolateral stimulation is in the one case 1.6 times, and in the other nearly twice, as great as that of homolateral stimulation.

Six photos similar to those of the last two figures are on hand. In two of them the left sciatic was stimulated, in the other four the right sciatic. With homolateral stimulation the deflection in the electrosympathicogram amounted to an average of 14.3 microvolts; with heterolateral stimulation the average reached more than double that amount, viz., 29.8 microvolts. In figure 5 the blood pressure rose from 65 to 120 mm. Hg in figure 6 from 75 to 145 mm. Hg.

In figures 7 and 8 are reproduced two curves of a cat, taken after the animal had been decerebrated according to the method of Sherrington. The right vagosympathetic lies on the electrodes. Note the results of heterolateral sciatic stimulation. In figure 7 the artificial respiration was arrested during the registration, while it was continued in figure 8. On stimulation the blood pressure in figure 7 rose from 55 to 125 mm. Hg; in figure 8, from 50 to 125 mm. Hg. What strikes us in the two figures is the marked prolongation of the latent period. Whereas, just before the decerebration, the latency had the normal length of a few hundredths of a second, it became ten times longer after decerebration, viz., 0.6 second. This is best determined in figure 7. In figure 8, however, although the respiratory oscillations interfere with accuracy of measurement, it is evident that the latent period lasts several tenths of a second. The delay cannot have been brought about by the stimulus being too weak, as in either case this

was even considerably stronger than usual, e.g., with the coils at a distance of only 2 cm. With homolateral stimulation a similarly prolonged latent period was found after decerebration. The circulation had been little, if at all, affected by decerebration, as may

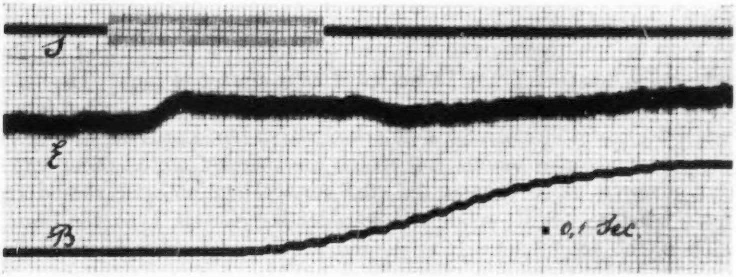

Fig. 7. Decerebrate cat. Artificial respiration stopped; left sciatic stimulaion; derivation from the right vagosympathetic; after decerebration the latent period was lengthened considerably; 1 scale-division of an ordinate equals 3 microvolts.

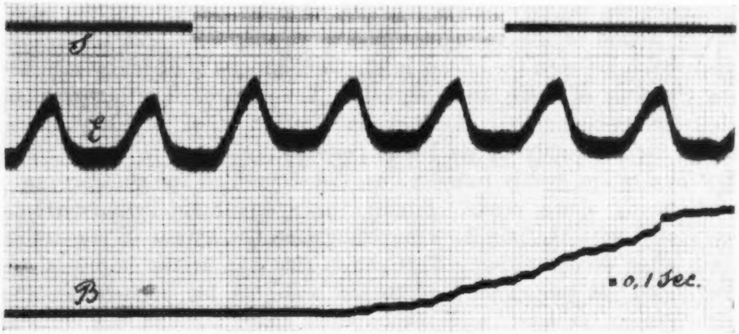

Fig. 8. Decerebrate cat with artificial respiration. The rest as in figure 7.

be concluded from the considerable rise in the blood pressure. How is this remarkable prolongation of the latent period to be accounted for? It seems most likely that the reaction of other fibers in the trunk of the sympathetic have come into play. After decerebration those

which innervate the pupil and nictitating membrane no longer respond promptly, if they respond at all, to sciatic stimulation. The fibers which innervate the salivary glands and a number of blood vessels of the head demand consideration. One of these two groups, or, which is more probable, the two groups together may have generated the action-currents registered.

That in our experiments a vasomotor action is transmitted by the cervical sympathetic may be accepted as a matter of fact. Throughout a large part of the body the blood vessels became constricted, which is proved directly by the marked rise in blood pressure and there is no reason for assuming that where the reaction of the other vasomotor nerves is so pronounced, the cervical sympathetic should remain inactive. Still, we do not consider it probable that the beginning of the action-currents is elicited by the vasomotor fibers of the sympathetic. In this connection it should be noted that the rise of the blood pressure manifests itself only more than two seconds after the onset of stimulation and persists for a considerable time after the stimulation has ceased and the elevation of the electrosympathicogram has passed off. The action of the vasomotor fibers in the cervical sympathetic has presumably a still longer latency and a longer after-effect than the action of the salivary nerves. Could we point out exactly the onset of the action of the vasomotor fibers, we might be able to sharply distinguish between the effects in the three groups of fibers by measuring the time of their latent periods. The electrosympathicogram of the salivary-secretion is distinguished from that of pupillary movement, etc., not only by its latent period but also by its shape. The first is a rather evenly running line, the second, on the contrary, presents many sharp peaks. Finally the sympathetic action-currents associated with the pupillary movements do not appear in registrable form after decerebration. This shows that the sciatic impulses are conducted via the spinal cord to the brain, as far cephalad as the diencephalon at least, and thence back again through the cervical cord to the sympathetic. This phenomenon substantiates in the main the theory advanced by the older physiologists.

This investigation, some of the results of which have been embodied in the curves presented above, may be extended in many directions. Mention has already been made of the application of thermionic valves to improve the method. In addition, different kinds of stimuli as, for instance, light might be employed. The paths followed by each type of stimulus in effecting reflex action-currents in the cervical

sympathetic might be precisely traced. Various species of animals might also be used and the influence of certain drugs studied. We hope to continue the research along these lines.

CONCLUSIONS

1. The functions of the cervical sympathetic can be studied by means of its action-currents.

2. The action-currents, accompanying the transmission of the stimulus effects to the eye, to the parenchyma of the salivary glands, and to the blood vessels in the head, are distinguished by their latent period. Upon stimulation of the sciatic nerve the electrosympathicogram of pupillary movement in the intact (undecerebrated) animal shows a latency of 0.04 to 0.06 second. For the same stimulus the electrosympathicogram of salivary secretion shows a latency of about 0.06 second. The transmission of the stimulus effects to the blood vessels takes probably much longer.

3. The latent period after a sound stimulus is about 0.18 second.

4. The latent period is shorter with strong than with weak stimuli.

5. The electrosympathicogram associated with salivary secretion may be distinguished not only by its latent period, but also by its shape, from that associated with pupillary movement. The first is almost a smooth line, whereas the second presents many sharp peaks.

6. By derivation from the united nerve-trunk, which contains the sympathetic, the vagus and the depressor fibers, a compound electroneurogram may be obtained, in which, besides the waves of the sympathetic currents, the rhythm of the heart-beat and the respiratory movements are visible.

7. The registrable reflex action-currents in the cervical sympathetic which induce pupillary movements seem to disappear after decerebration. This indicates that peripheral stimulus effects, e.g., from a paw, first take their way to the brain, as far cephalad as the diencephalon at least, and thence back again through the spinal cord to the cervical sympathetic. The reflex action-currents in the sympathetic, which cause salivary-secretion and vasoconstriction, persist after decerebration.

8. Apnea (acapneic shock) may weaken or even abolish temporarily sympathetic function.

9. Reflex action through the cervical sympathetic may be induced by various types of stimulus, viz., *thermal*, applied to the skin e.g. of the gluteal region and abdomen, *auditory* (loud sounds), *electrical* applied e.g. to the sciatic nerve.

10. Stimulation of a sciatic nerve evokes stronger action-currents in the heterolateral than in the homolateral cervical sympathetic.

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THE TOXICITY OF THE ALCOHOLIC EXTRACT OF OX BILE WHEN FED TO WHITE RATS

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The toxicity of ox bile for white rats was observed while various body fluids were being tested for their vitamine-A content (1). The experiments reported here were later carried out to determine, if possible, the toxic substance.

According to our present knowledge, bile is both an excretion and a secretion of importance in the work of the intestines. Bile introduced parenterally is toxic, but taking bile by mouth is reported to have no deleterious effects. Evidently the toxic constituents of the bile are normally not absorbed in sufficient quantities from the stomach and intestines. Regurgitation of bile into the stomach is a normal physiological phenomenon (Boldyreff, Carlson). Mastrosimone reports four cases of anastomosis between the cystic duct and the stomach. All these patients seemed to be in the best of health even after periods of three and four years (2). Groll (3) has shown that high concentrations of bile retard the activation of salivary amylopsin. He quotes Ringer as stating that bile has a retarding influence also upon the pepsin activity. Pannet and Wilson (4) observed in cats and man that the addition of a small quantity of sodium taurocholate to the diet caused an abnormally rapid evacuation of the stomach. It is also reported that bile in some way aids the normal movements of the intestines. If the bile or the bile acids retard the activity of the digestive ferments in the stomach and cause a rapid emptying of the stomach this may account for the loss of weight in animals given a diet containing bile extracts. But this does not account for the fatal toxicity of these extracts.

Experimental. Ox bile in quantities of 1000 cc. were evaporated to dryness on a water bath and extracted with ether and alcohol. The two extracts were combined. The ether and alcohol were evaporated and the dry residue from each 1000 cc. of bile added to 200 grams of a purified synthetic diet. This purified diet consisted of:

	per cent
Casein	20
Fat (crisco)	25
Salt mixture	4
Yeast extract	5
Corn starch	50

Roughage in form of agar agar to equal 4 per cent of entire diet.

The diet was highly pigmented with biliverdine and rather gummy in nature. Ten grams of this diet were given daily but not all of the food was eaten. It is difficult to estimate carefully the amount which was eaten each day, as two or three grams in weight were lost by evaporation. It is believed that between three and four grams of food were eaten from each portion.

The animals used were white rats. They had been raised in the laboratory from healthy stock. When they became one month or five weeks old, they were taken from the mother and put upon a purified synthetic diet which was theoretically adequate in all respects. After a period of from ten days to two weeks they had shown a distinct gain in weight. They were then put upon a standard vitamine-free diet. After another period of about two weeks, these rats were showing a cessation of growth or in many cases a distinct loss in weight. The purpose of this preliminary feeding or dieting was to make sure the animals were reactive to the presence and to the absence of the vitamine-A. The rats were then started upon this special bile extract diet.

1. Results from feeding a diet containing the combined ether and alcoholic extracts of bile. The eight rats put upon this experimental diet were about two months old and had ceased gaining in weight or had been losing slightly on the vitamine-A free diet. Nevertheless each rat appeared strong and vigorous at this time. On the vitamine-A free diet plus the bile extract one animal was found dead within one-half day. Two more died during the first day, and four more within the next two days. The last animal died on the sixth day. All the rats that lived longer than a day showed distinct loss in weight. Upon autopsy the stomach and intestines were found filled or partially filled with food. In six cases the lungs were hemorrhagic. There was bloody urine present in the bladder in five cases. In one rat the urine was not bloody. In the others the bladder was found empty. The mucosa of the stomach was eroded in some cases. All the abdominal viscera appeared congested.

In order to see whether this particular diet would act in a similar fashion upon larger rats that had not previously been subjected to

vitamine-A free diet, four robust rats weighing slightly over 100 grams were taken directly from the stock and fed each day the combined ether and alcoholic extract diet. Within five days all animals had died. Three were dead before the fourth day and the last died on the fifth day. The weight of each rat decreased rapidly during this short period. It was noticed that these animals soon became extremely quiet, rarely moving about in the cage. Later they became quite cold, and their legs and feet stiff. Two showed bleeding from the nose. In three the lungs were hemorrhagic. There were erosions in the gastric mucosa in three rats. The urine was bloody in those cases in which the bladder was not empty.

2. Results from feeding, (a) standard diet plus the ether extract of bile; (b) standard diet plus the alcoholic extract of bile; (c) standard diet plus the water extract of bile. New extracts were made. The ether extract was alone added to the standard vitamine-A free diet. The alcoholic fraction was added to another portion of the same diet. The standard basic diets were the same in every respect as described above. The animals were prepared in the same fashion as those first described. They were tested for their susceptibility to the vitamine-A with the hope that the presence or absence of vitamine-A in bile could be detected, if the extract was non-toxic.

Six rats were put upon the ether extract diet. At the end of two weeks all animals were living and well. All save one had lost some in weight but they showed no symptoms similar to those that had died on the combined extracts of bile.

Six rats were put upon the alcoholic extract diet. At the end of four days three animals were dead; after five days two more were dead, and at the end of seven days the last animal was dead. These animals became quiet and showed a fall in body temperature. Upon autopsy, the gross pathology was similar to that found in those dead from the combined extract diet. Pleural edema was found in four cases.

Four animals were put upon the water bile extract diet. This diet was rather light green in color and less gummy. At the end of two weeks all animals were alive and showed no toxic signs. Each had, however, lost some in weight.

3. Results from feeding standard diet plus pure taurocholic acid. Two rats were fed daily 0.05 gram each of pure taurocholic acid. This quantity was believed to be equal to the amount each rat would receive by eating 3 or 4 grams of bile extract diet. This crystalline substance was mixed with a small ball of standard vitamine-A free diet. After this

had been eaten, a larger quantity of diet was given. In this way the weighed portion of taurocholic acid was consumed each day by each rat. At the end of two weeks the rats showed no difference in appearance or in conduct from that observed at the beginning of the experiment. They had lost very little in weight during this time.

The post-mortem findings (purpura, internal hemorrhages) are suggestive of bile salt intoxication. This would explain the harmlessness of the ether extract of bile, since the bile salts are insoluble in ether. But it does not explain the non-toxic effects of the water extract, for the bile salts are even more soluble in water than in alcohol. Nor does it explain the negative results of adding the taurocholic acid to the diet. However, this might be merely a quantitative factor. The percentage of bile salts in the bile used was not determined. Hence we do not know how much bile salts were ingested per day in the diets containing the water and alcoholic bile extracts. There may be alcohol soluble toxic substances in the bile other than the two chief bile salts. This must be determined by further experiments.

SUMMARY

1. In the quantities fed, the combined ether and alcoholic extracts of ox bile are fatally toxic to white rats. Larger and more vigorous rats taken directly from the stock and put upon such a diet die with toxic symptoms just as quickly as younger rats that have previously been upon purified synthetic diets. All animals upon this experimental diet died within six days.

2. The ether extract of bile added to the standard synthetic diet is not toxic to white rats. All animals upon such a diet were living and well at the end of two weeks.

3. The alcoholic extract of bile added to a standard synthetic diet is fatally toxic to white rats. All animals upon such a diet died within seven days.

4. The water extract of bile added to a standard synthetic diet is not toxic to white rats. There appeared to be very little change in the animals put upon this diet, even at the end of a two weeks' feeding period.

5. Pure taurocholic acid fed at the rate of 0.05 gram per day is not toxic to white rats. At the end of two weeks these animals appeared normal.

I wish to thank Doctor Carlson for suggestions in the course of the work; Doctor Sugata of the Department of Physiological Chemistry for the pure taurocholic acid used in this experiment; and Doctor F. S. Koch for advice as to the amount of this acid to be fed daily.

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STUDIES ON THE PATHOGENESIS OF TETANY

II. The Mechanism Involved in Recovery from Parathyroid Tetany

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In previous reports of this study on the pathogenesis of tetany by Dragstedt (1) and Dragstedt and Peacock (2), it was found that completely parathyroidectomized dogs could be kept alive indefinitely without developing tetany, if kept on a special carbohydrate diet. The most successful diet used consisted of white bread and milk ad libitum and lactose in amounts varying from 50 to 125 grams per day. Such diets bring about a practically complete suppression of bacterial proteolysis in the intestinal tract, the feces become liquid, odorless, acid to litmus, and the intestinal bacteria changed from the ordinary mixed flora into one consisting predominantly of streptococci and Gram positive lactic acid bacilli. The diet was usually given for one or two weeks before the operation and the change in the character of the feces well established. Both the thyroid and parathyroid glands were widely removed, the attempt being to make the operation as complete as possible. The animals quickly recovered from the operation and appeared entirely normal. Some developed a loss of appetite and refused food on the third or fourth day and with this anoxeria a cessation of bowel movements. Shortly thereafter transient mild tremors and spasticity usually were manifest. This was relieved if the milk and lactose solutions were administered by stomach tube and the animal prevented from vomiting. The relief usually followed the discharge of feces. Depending upon the degree to which the animal could be made to take the diet and the bowels kept moving, all evidences of tetany or depression were absent. If the special diet was continued for five or six weeks, at the end of this time the animal could take the ordinary stock diets of bread, meat, table scraps and vegetables, without developing tetany or depression. Previous to this time such a diet would

throw the animal into violent convulsions or depression, and if continued the animal would invariably die. This fact is perhaps the best evidence that the entire parathyroid apparatus has been removed and is certainly more reliable than failure to find parathyroid tissue at autopsy. The majority of animals require five or six weeks to readjust themselves to the loss of the parathyroid glands. Old dogs may make this readjustment sooner, whereas young dogs require a considerably longer period.

The survival of completely parathyroidectomized dogs when placed on such diets as inhibit proteolysis and putrefaction in the intestine, has been interpreted to indicate a normal detoxicating function of these glands. The tetany or depression which appears in untreated animals is the expression of an intoxication, resulting from the accumulation of toxic products in the blood which are normally removed through some functional activity of the parathyroid glands. It is evident that the chief source of the toxic materials in otherwise healthy, non-pregnant animals is the gastro-intestinal tract and consists most probably of the highly toxic protein split products resulting from the proteolytic action of the intestinal bacteria. That tetany poisons may have their origin elsewhere than through bacterial action in the intestine is indicated in experiments to be reported later. However, their importance may be readily estimated when it is seen that parathyroidectomized animals do not develop tetany and may survive when intestinal toxemia is checked, but do develop it otherwise, unless of course these poisons are removed from the blood after absorption as was done by Luckhardt (3).

There are several possibilities of parathyroid function. The parathyróid cell may remove these toxic substances from the blood and neutralize or destroy them. Against this view is the small size of the glands.

The parathyroid cells may provide a hormone which neutralizes the toxic products in the blood or which stimulates some other organ to such detoxication.

The parathyroid cells may provide a hormone which regulates the functions of the gastro-intestinal tract, and in the absence of which there occur derangements in motility, secretion and absorption. Under such pathological conditions the production of intestinal poisons might be so great as to break down the protective functions of the intestinal mucosa and the liver. It is a fact, demonstrated by Carlson (4) and Keeton (5) that in parathyroid tetany there is a marked depression

in gastro-intestinal motility and secretion, and in severe cases a complete paralysis. There can be no doubt that such alimentary disturbances make the condition worse once the tetany has developed, since it is well known that in paralytic ileus there is a marked increase in the production and absorption of poison from the intestines. However, the gastro-intestinal disturbance is probably the result of the intoxication rather than the cause, since the parathyroidectomized dogs on the dietary regime where the intoxication did not occur, gave no evidence of digestive disorder. Peacock and Dragstedt (6) found that the gastric secretion in those animals was entirely normal. It is probable that the pancreatic secretion and intestinal absorption are likewise normal, since the dogs gain in weight with ordinary amounts of food. There remains, of course, the possibility that the permeability of the intestinal mucosa to the poisons in the intestines is increased in the absence of a parathyroid hormone.

Mechanism of the readjustment of the body to the loss of the parathyroid glands after five or six weeks' dietary treatment. Until the mechanism of parathyroid function is established, it will be difficult to determine the mechanism of the body readjustment to the loss of these glands. On the basis of analogy with other endocrine organs and some direct evidence, it is possible that minute remnants of parathyroid tissue left behind at the operation, and which were immediately unable to prevent tetany, may have undergone sufficient hypertrophy to take care of the amount of toxic materials in the blood under ordinary conditions.

It is possible that the tissues, particularly the nervous system, may have developed a tolerance or immunity to the tetany poisons, so that although these are present in the blood, they no longer produce symptoms.

It is possible that some other organ has taken over the function of the missing tissue and is acting vicariously for it. This possibility is particularly indicated in the following experiment in which tetany recurred in a completely parathyroidectomized dog following the production of a liver injury by the administration of phosphorus.

Dog 25. Large male adult, weight 20 kgm.

February 26, 1922. Diet of white bread and milk ad lib. and lactose 44 grams per day, in solution.

March 6, 1922. Feces liquid, odorless, acid to litmus, fecal bacteria predominently acid uric. Thyroid and parathyroid glands were widely removed.

March 7–April 2. Diet as above. Condition excellent. No evidence of tetany or depression.

April 2. Animal given stock diet of bread, meat and vegetables.

April 3–April 11. No evidence of tetany or depression.

April 12, 3:00 p.m.; 0.12 gram of yellow phosphorus suspended in olive oil given by stomach tube.

April 13. Condition good. No untoward symptoms.

April 14, 4:00 p.m. The animal developed a severe attack of tetany which lasted until 6:00 p.m. The attack began with fibrillary tremors in the temporal muscles and a gradually increasing hyperpnea. At 5:00 p.m. the tetany was extreme, the animal showing tonic and clonic convulsions, marked salivation, and several times complete cessation of respiration due probably to a spasm of the glottis. Six hundred cubic centimeters of Ringer's solution were given by intravenous injection. Recovery complete at 7:00 p.m.

April 15. Animal depressed, showed some fibrillary contractions about temporal muscles. Urine contained phosphorus (Scherer's test) but no albumin or sugar. One hundred and sixty grams of lactose, 1000 cc. of milk and 500 cc. water given by stomach tube.

April 16. Animal depressed, fibrillary tremors in temporal muscles, slight clonic convulsions. Seven hundred cubic centimeters of Ringer's solution given intravenously; 400 cc. of milk and 400 cc. of 20 per cent lactose solution given by stomach tube.

April 17. Depression slight, fibrillary tremors and few clonic contractions of jaw muscles. Eight hundred cubic centimeters of milk and 400 cc. of 20 per cent lactose solution given by stomach tube.

April 18. Animal lively, good condition, no evidence of tetany. Diet white bread, milk and lactose.

April 19–24. Diet as above; no evidence of tetany.

April 25. Animal ate about 2 pounds of meat.

April 26. Clonic contractions of jaw muscles, causing rapid, repeated snapping of jaws.

April 27. Diet of white bread, milk and lactose. No evidence of tetany.

April 28–May 2. Diet as above; no evidence of tetany.

May 3. Animal given usual stock diet. There has been no further evidence of tetany on this diet and animal is in good condition at present time, April, 1923.

The tetany which recurred in this animal following the administration of phosphorus could not be distinguished from a severe attack of typical parathyroid tetany. The administration of 0.24 and 0.48 gram of yellow phosphorus to normal dogs of the same weight had no noticeable effect.

It is well known that phosphorus poisoning produces an intensive injury to the liver, indicated by the delayed excretion of phenol-tetrachlor-phthalein, fatty infiltration, decrease in blood fibrinogen, increased excretion of ammonia, and increased protein destruction with incomplete deamidization of certain of the amino-acids. There is an increase in the non-protein nitrogen in the blood and the urinary nitrogen is greatly increased.

The reappearance of tetany in this animal following the phosphorus poisoning perhaps indicates a direct relation between the liver and the parathyroid glands, and a probable similarity in function. It is quite possible that the liver may have taken over the function of the missing parathyroid glands, to the extent that tetany was prevented although the animal was given a mixed stock diet. This would involve a detoxicating function which is known to be possessed by the liver for many different poisons. With the injury to the liver, however, this vicarious action might very probably be lost and the animal returned to the condition immediately following the parathyroid extirpation.

The experiments of Koch (7) and of Paton and his associates (8) have led these workers to conclude that guanidine is the substance responsible for tetany following parathyroidectomy. The theory is advanced that guanidine represents a product of endogenous protein metabolism, which is normally broken down through the function of the parathyroid glands. A precursor to guanidine may be cyanamid. The chief evidence for this theory lies in the reported isolation of abnormal amounts of guanidine and methyl guanidine from the urine and blood of parathyroidectomized dogs. The intravenous injection of these substances in the cat, rat and rabbit was stated to produce symptoms indistinguishable from those of parathyroid tetany. Paton and Findlay conclude that the symptoms of guanidine injection bear the same relationship to those of tetania parathyreopriva that those following the administration of diphtheria toxin bear to the symptoms of true diphtheria.

Our experiments contradict the theory of the endogenous origin of the tetany toxins since it is difficult to see how the special diets used could alter the endogenous metabolism and yet in our experiments tetany was prevented. It seems highly improbable that guanidine or any other *one* toxic substance is responsible for the diverse and protean symptoms seen in experimental tetany. It must be emphasized that depression is just as characteristic of parathyroid removal as is tetany, and in some species is the only manifestation. Dragstedt (9) found that the toxicity of the content of the normal and obstructed intestine depends upon a number of toxic protein derivatives, formed chiefly through the proteolytic activity of the intestinal bacteria. Histamine was isolated by Gerard (10) as one of the most important and active of these substances. Since the diets which prevent the appearance of parathyroid tetany also prevent the production of these intestinal poisons, it seems most probable that the poisons bear an etiological

relation to the tetany symptoms. This is especially indicated in the following experiment in which an amount of these poisons, too small to affect a normal dog, caused tetany in a dog whose parathyroid glands had been long removed.

Dog 28. Large adult female; weight 18 kilos.

February 10, 1922. Diet white bread and milk ad lib. and lactose 60 grams per day.

March 6. Feces odorless, acid to litmus, fecal bacteria predominantly aciduric. Thyroid and parathyroid glands widely removed.

March 7–9. Condition good, no evidence of tetany.

March 10. Slight spasticity and tremor. Four hundred cubic centimeters of milk and 400 cc. lactose solution (23 per cent) given by stomach tube.

March 11–April 16. Condition good; no evidence of tetany or depression.

April 17. Stock diet of meat and vegetables substituted for special diet.

April 18, 1922 to January 22, 1923. Condition excellent; no evidence of tetany at any time.

January 23, 1923. Condition good. Weight 20 kilos. Two cubic centimeters of material collected from an obstructed intestinal loop in a dog was injected intravenously. In 6 minutes occurred violent expulsion of feces and considerable depression. In 30 minutes recovery seemed complete. In 4 hours marked *spasticity* developed in the legs together with *fibrillary tremors* in the temporal muscles. There were no convulsions.

For comparison the same intestinal material was injected into normal dogs. The same dose, namely, 0.1 cc. per kgm., had no effect whatever. A much larger dose, 0.66 cc. per kgm., caused defecation, vomiting and depression, but no tetany. The much greater susceptibility of the parathyroidectomized dog to these intestinal poisons may indicate that a part at least of the function of the parathyroid glands has to do with the protection of the body against poisons of this nature.

Susceptibility of parathyroidectomized dogs to guanidine, methyl guanidine, trimethylamine and histamine. There are many suggestions in the experimental literature as to the nature of the toxic agent in parathyroid and idiopathic tetany. For the most part the substances considered have been found in the body tissues or excretions and have the property of causing convulsions similar to those of parathyroid tetany when injected into normal animals. Curiously, substances that cause a primary depression on injection have been rejected on that account, although it has long been known that many parathyroidectomized animals show depression continuously from the start with no tetany at any time. Jacobson (11) concluded that ammonia was the toxic agent, but the later experiments of Carlson and Jacobson (12) caused these workers to conclude that an increase of ammonia in the blood was not

the cause of tetany. Berkeley and Beebe (13) suggested xanthine; Biedl suggested histamine. Koch (7) and Paton and his associates (8) have strongly supported the theory that guanidine and methyl-guanidine are the toxic agents. Koch found in addition to guanidine and methyl-guanidine, trimethylamine, histamine, choline and neurine in the urine of parathyroidectomized dogs.

In studying the relation of these various chemicals to the symptoms following parathyroidectomy, it must be considered that their effect

TABLE 1

Effect of guanidine hydrochloride on normal dogs and dogs that have survived a complete parathyroidectomy

NORMAL DOGS	DOSE PER KILOGRAM (INTRAVENOUS)	EFFECT
	gram	
1	0.050	No untoward symptoms
2	0.050	No untoward symptoms
3	0.070	Vomiting, sneezing; no tetany
4	0.080	Vomiting, sneezing, groaning; no tetany
5	0.100	Vomiting, sneezing, depression, slight tremors
6	0.106	Vomiting, sneezing; no tremors
7	0.150	Marked depression; slight tremors, vomiting

PARATHYROIDECTOMIZED DOGS	TIME SINCE OPERATION	DOSE PER KILOGRAM (INTRAVENOUS)	EFFECT
	months	*gram*	
1	9	0.019	Uneasy; no depression; no tetany
2	4	0.020	No abnormal symptoms
3	6	0.021	Depression; vomiting; no tetany
4	4	0.030	Spasticity, tremors, vomiting
5	10	0.040	Vomiting, depression, no tremor
6	4	0.050	Marked spasticity, tremors, vomiting

on injection might be quite different in animals whose parathyroids have been removed and in normal animals. This would be true if the parathyroid glands had a functional relation with the nervous system or other tissues aside from the detoxicating function evidenced by these studies. Fortunately, the survival of completely parathyroidectomized dogs, where intoxication from the intestine is checked by diet, without symptoms of tetany, provides an opportunity for testing the effect of many of the above substances on animals without parathyroids but who appear normal in other respects. The following tables show the

TABLE 2

Effect of methyl-guanidine hydrochloride on normal dogs and dogs that have survived complete parathyroidectomy

NORMAL DOGS	DOSE PER KILOGRAM (INTRAVENOUS)	EFFECT
	gram	
1	0.040	Sneezing, coughing. No tetany
2	0.050	Sneezing, coughing. No tetany
3	0.100	Sneezing, weakness, ataxia; no tremors; no tetany
4	0.100	Sneezing, weakness, ataxia, marked depression

PARATHYROIDECTOMIZED DOGS	TIME SINCE OPERATION	DOSE PER KILOGRAM (INTRAVENOUS)	EFFECT
	months	*gram*	
1	4	0.020	Tremors, spasticity, vomiting
2	13	0.057	Vomiting, no tremors, no depression
3	3	0.050	Vomiting, spasticity, tremors, vomiting
4	2	0.060	*Tremors*, salivation, depression, "chattering," blepharospasm, enophthalmos, spasticity

TABLE 3

Effect of trimethylamine hydrochloride on normal dogs and dogs that have survived complete parathyroidectomy

NORMAL DOGS	DOSE PER KILOGRAM (INTRAVENOUS)	EFFECT
	gram	
1	0.050	Slight weakness, depression; no tetany
2	0.060	Spasticity, defecation, weakness
3	0.060	Vomiting, defecation; no tremors; no spasticity
4	0.075	Vomiting, defecation, weakness; no tetany
5	0.100	Depression marked, no tremors, no spasticity

PARATHYROIDECTOMIZED DOGS	TIME SINCE OPERATION	DOSE PER KILOGRAM (INTRAVENOUS)	EFFECT
	months	*gram*	
1	4	0.020	Gasping, no tremors, no depression
2	12	0.040	Prostration, gasping, salivation, quick recovery
3	6	0.050	Prostration, profound weakness, salivation

effects produced by gradually increasing doses of various toxic chemicals in normal dogs and in dogs whose parathyroid glands have been absent for varying periods.

The experiments bring out the following significant points:

1. The parathyroidectomized dogs are much less resistant to guanidine, methyl-guanidine, trimethylamine, histamine, and the various intestinal poisons than are normal dogs.

2. The predominant effect on normal dogs of guanidine, methyl-guanidine, trimethylamine and histamine, in the doses used, was in the direction of depression, with vomiting and defecation as characteristic symptoms. A typical attack of tetany such as occurs in the parathyroidectomized dog was never observed.

TABLE 4

Effect of histamine hydrochloride on normal dogs and dogs that have survived complete parathyroidectomy

NORMAL DOGS	DOSE PER KILOGRAM (INTRAVENOUS)	EFFECT
	gram	
1	0.0001	Defecation, no tremors, no depression
2	0.0003	Defecation, no tremors, no depression
3	0.0005	Defecation, vomiting, depression, no tremors
4	0.0005	Defecation, vomiting, depression, no tremors
5	0.0010	Defecation, depression, no tremors

PARATHYROIDECTOMIZED DOGS	TIME SINCE OPERATION	DOSE PER KILOGRAM (INTRAVENOUS)	EFFECT
	months	*gram*	
1	4	0.0001	Vomiting, tremors, defecation
2	12	0.0002	Immediate wild excitement. *Defecation*, urination, severe prostration
3	6	0.0002	Great restlessness, vomiting. Defecation, marked and long-continued depression

3. Guanidine and methyl-guanidine may produce symptoms of tetany (spasticity, tremors, salivation and hyperpnea) in parathyroidectomized dogs in doses that have little or no effect on normal dogs.

4. Recently parathyroidectomized dogs are less resistant to the various poisons used than are animals who have recovered for long periods. There is an increasing resistance.

DISCUSSION

The experimental evidence seems most in harmony with the theory that the parathyroid glands form a part of the detoxicating mechanism of the body. The evidence is well established that the endothelial cells and liver possess such function and it is possible that the parathyroid glands perform their function as a part of this system. The action may be indirect, brought about through the medium of a parathyroid hormone stimulating this function of the liver and the endothelial cells. The intestinal mucosa hinders intoxication from the intestines probably chiefly because of its capacity for selective absorption. This protective action is not adequate, however, when the parathyroid glands are removed and it is most probable that under normal conditions there is a continued absorption of intestinal poisons which are cared for by the detoxicating system above described.

The readjustment which occurs following complete parathyroidectomy involves a gradually developing and progressive increase in resistance on the part of the body to the tetany poisons. This readjustment does not necessarily involve a decrease in the permeability of the intestinal mucosa to the tetany poisons since the increasing resistance can be demonstrated by injecting these poisons intravenously. It is not probable that the nervous system has become more resistant to these poisons although this point cannot be considered settled. The most probable explanation in view of the experimental evidence to date is that the liver and perhaps other tissues have developed an increased detoxicating ability and have taken over in part the function of the missing glands.

None of the various chemicals produced typical parathyroid tetany when given intravenously in normal dogs. The symptoms were chiefly in the direction of depression with severe gastro-intestinal disturbance. Guanidine and methyl-guanidine were more apt to induce tetany in the recovered parathyroidectomized dogs and in smaller doses than occasioned depression in normal animals.

There is reason to believe that the parathyroidectomized animal has a decreased resistance to a great number of different toxic chemicals. It is obvious that the symptoms displayed by various animals of the same species and especially so in different species would depend upon the pharmacologic activity of the most active poison or poisons absorbed and this might well be different under varying conditions. The chief source of these poisons in the normal non-pregnant dog is the gastro-

intestinal tract and the predominant poisons there depend upon the chemical composition of the diet and the nature and activity of the intestinal bacteria.

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THE CIRCULATORY RESPONSES OF MAN TO A SUDDEN AND EXTREME ANOXEMIA

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An acute and extreme oxygen want, uncomplicated by other stimulating factors, was obtained by breathing pure nitrogen. The nitrogen gas, saturated with water vapor, was delivered from a large rubber bag through a Larsen automatic respirometer (1) on which valves were so arranged that the subject of experimentation was suddenly given the gas by the turning of a valve during the pause at the end of the respiratory expiration. By the same means he was later as quickly returned to fresh air.

The latent period and the extent of the respiratory and pulse rate responses to the conditions of this method of producing anoxemia have been determined by Lutz and Schneider (2).

The purpose of this paper is to consider more fully the circulatory reactions, particularly those of the arterial blood pressure, the hand volume, and the volume of blood flow through the hand; and to establish curves to show the rate and degree of change. The effects of the anoxemia will be considered not only during the development of the anoxic condition but also through the period in which normal conditions were being reëstablished in the body.

In the experiments the subject was permitted to breathe the nitrogen until unconsciousness was impending, at which time the eyes tended to converge and sometimes the pupils to dilate. It was necessary for the observer, who was responsible for the welfare of the subject, to be familiar with and alert to the symptoms of anoxemia, as ordinarily the symptoms became more pronounced for a few seconds after fresh air was given. This was, of course, due to the fact that the lungs were so filled at the close of the nitrogen period that the first breath of air only slightly diluted the nitrogen in the alveoli. Consequently four or five deep breaths of air were required to reëstablish the normal

alveolar oxygen pressure. In our experience none of the subjects fainted; although a number of persons were carried so far that muscles of the head, arms or legs twitched in the early stages of asphyxial decrease of muscle tone. In some instances the breathing began to slow and even stopped in several cases, but by slapping the back of the subject a deep inspiration was immediately obtained. It was our custom to prompt the subject, on restoring him to air, to take deep breaths until he had taken five or six. Practically all subjects reported that the symptoms of breathing nitrogen were not unpleasant. In this experience one feels quite as though he were undergoing nitrous-oxide anesthesia. There is no discomfort; during the early stages of the process the voices round about are clear and distinctly heard and the field of vision wide and bright. Soon, however, the voices begin to recede, becoming gradually further and further away, the field of vision likewise grows gradually less bright and narrower and then all fade away. On the return to fresh air there is a feeling of dizziness, with ringing in the ears, sometimes double and dim vision, and again the far-away voices. The vision gradually clears and voices come to be one and that associated with the operator at one's elbow.

In our work a subject underwent this form of anoxic anoxemia four to six times in succession. An interval of rest of 5 minutes was allowed between each of the tests. Before, during and after the nitrogen period the pulse rate was recorded on a Mackenzie polygraph, the blood flow determined by the Hewlett-Van Zwaluwenburg (3) method, the hand volume with the ordinary plethysmograph, and the arterial blood pressure with a Tycos sphygmomanometer by the auscultatory method. In each test a single observer watched and recorded only one kind of reaction. The systolic and diastolic arterial blood pressures were determined separately in different experiments. In one pair of tests only the systolic pressure was recorded and in the second pair only diastolic pressures. With an experienced operator it was possible to obtain determinations of either systolic or diastolic pressure alone at the rate of one determination in 10 seconds.

The length of the period of breathing nitrogen ranged from 47 to 112 seconds, with a mean duration of 82 seconds. There were 17 men and women who served in 164 tests. From these data we have constructed the typical or ideal curves for the pulse rate and for systolic, diastolic and pulse pressures. The curves were established in a somewhat unusual way. It was an easy matter to determine the first part of each curve, in that we had only to determine the means for the data at

regular intervals; but for the curve toward the end of the nitrogen period this method could not be used, because the termination of nitrogen breathing ranged between 47 and 112 seconds. When the number of cases began to be reduced by the falling out of some, the means calculated in the usual way gave an irregular curve. Since the physiologic responses in each case had reached their limit at the time the nitrogen was cut off, as was indicated by impending unconsciousness, we assumed that it would be permissible to use as the end value for our generalized curves the mean of the last readings of the nitrogen period of all cases.

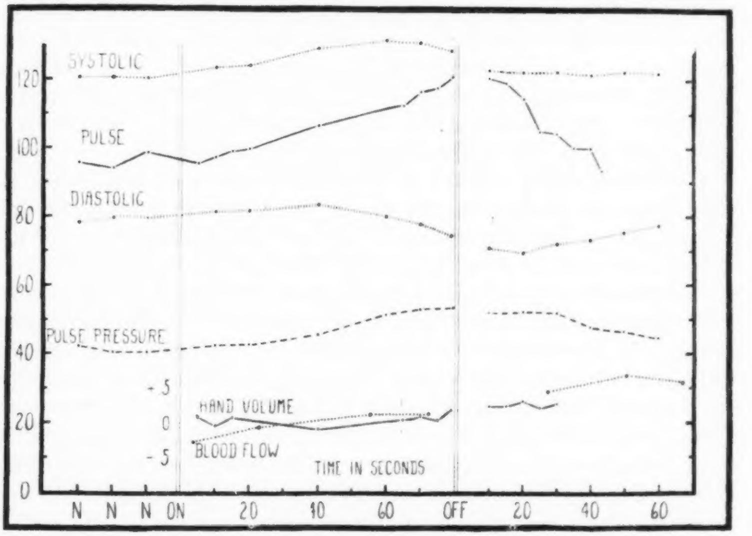

Fig. 1

Then means were determined for all cases for two and three equal intervals of 5 and 10 seconds back from the time nitrogen breathing ceased. The mid-point for the curves was next determined by recording the value of the mid-point of the individual curves and from these was computed the mean mid-point. The curves shown in figure 1 were plotted from the means so obtained. The pulse rate curve was determined from 56, the arterial systolic pressure curve from 84 and the diastolic pressure curve from 82 tests.

From the data summarized in the curves of figure 1 it is evident that the heart accelerates very quickly under the stimulus of acute anoxemia.

Lutz and Schneider found that 44 per cent of their cases gave a latent period, in the response to the decreased oxygen, of not more than 10 seconds; and that 22 per cent gave one of 15 seconds. Our generalized curve has a fleeting psychic rise in the pulse rate, the occasion of which was the starting of the breathing of the nitrogen. Omitting this psychic rise the means for the pulse rate were found to be, normals 95.8 and 94.1, 5 seconds of nitrogen 95.6, 10 seconds 97.2, 15 seconds 99. Hence the anoxemia was effective within 10 seconds and from then on the heart accelerated smoothly and steadily until at the close of the period of nitrogen breathing the mean rate was 120.9. The retardation of the return to normal was made almost as smoothly and more quickly than the acceleration. The first mean after "off" was 120.7 and for each succeeding 5-second interval was 119.1, 114.5, 105, 104, 100.1, 100, 99.8. The retardation was rapid during the first 20 seconds and slower thereafter. A slight after-effect is indicated.

The systolic blood pressure in 84 tests showed an average rise of 11 mm. Hg, in one instance the rise was 37 mm., the next greatest rise was 24 mm. and in only one was there no rise. The generalized curve for the systolic pressure is not as good as that for the pulse rate because toward the close of the period of nitrogen breathing the pressure was still rising in some and falling in other tests. The most common condition is represented by the generalized curve of figure 1, in which a gradual rise begins early, within 10 seconds, and reaches a maximum some seconds before the end of the nitrogen period. In 17 tests the systolic pressure continued to rise until the close of the nitrogen period; in 11 tests it rose to a maximum and then maintained a level; while in 57 it rose for a time and then fell, in some slowly and in others rapidly.

The after-period shows a rapid return to normal. The last determination of the mean systolic pressure in the nitrogen period was 128.3 mm.; immediately after giving fresh air it was 123.1 mm., and 10 seconds later, 122.1 mm. The normal of the pre-nitrogen period was 120.4 mm.

The diastolic pressure changes as brought out by the curve of means in figure 1 were obtained from 82 tests. The first effect of this rapidly produced form of anoxic anoxemia was to cause a slight but definite rise in the diastolic pressure which rose from a pre-nitrogen period normal of 79.3 mm. Hg to 83.1 mm. by the middle of the nitrogen period. Some rise was already present by the end of the first 10 seconds of the nitrogen period. However, emphasis should not be placed on this as an evidence of an early response of the vasomotor center to the fall in

the arterial blood oxygen pressure as we were unableto entirely eliminate the possibility of a psychic effect. The diastoli c pressure began to fall slowly shortly after the middle of the nitrogen period and continued to decrease gradually, not only through the remainder of this period, but on into the post-nitrogen period for as much as 20 seconds. Then there occurred a gradual recovery of vasomotor tone which restored the pressure to normal about 70 seconds after the breathing of nitrogen was discontinued.

The tests did not all conform to the generalized curve of figure 1. In 4 tests the diastolic pressure rose throughout the entire nitrogen period; in 13 it remaied constant; while in 65, about 80 per cent, the initial rise and the later fall in pressure occurred.

The pulse rate and the arterial blood pressure reactions to the acute and extreme oxygen want caused by breathing nitrogen for a period of about a minute are quite like those showh by men while rebreathing 52 liters of air for a period of 25 to 30 minutes, during when time the oxygen is reduced to between 6 and 7.5 per cent. Schneider and Truesdell (4) have determined the generalized or composite curves for 148 non-fainting cases under the latter conditions and found that the pulse rate accelerated from a mean of 81.8 to 110.7 at 7 per cent oxygen; the systolic pressure rose from 121.2 to 137 mm. at 7 per cent oxygen; and the diastolic pressure rose from 81.5 mm. to 87.9 mm. at 13 per cent oxygen, and then gradually fell to 74.6 mm. until 7 per cent oxygen was reached. It was also found that in approximately 33 per cent of the cases the systolic pressure began to fall before the subject of experimentation became inefficient.

While the circulatory reaction to these two varieties of a progressively developing anoxemia are quite similar it has been found that a given individual will not as certainly react twice in exactly the same degree and in the same manner to the anoxemia induced by breathing pure nitrogen as to the slower anoxemia caused by the rebreathing of 52 liters of air. Furthermore the rapidly developed anoxemia of nitrogen breathing gives too short a time for accurate observations on the sensory and mental changes that occur. Individuals differ in their capacity to respond to and endure low oxygen. These differences are more clearly brought out with the slowly developed anoxemia and almost not at all by the rapidly developed anoxemia that brings on unconsciousness within 1 or 2 minutes.

The fall in the diastolic pressure which was so constantly present during the breathing of nitrogen made it desirable to look for other

evidence of vasodilatation. For this purpose the hand volume was studied with the plethysmograph for 12 subjects in 52 tests. No regular change in a single direction was observed. The mean change in cubic centimeters for all tests was calculated and has been plotted in a curve in figure 1. It shows that practically no change occurred in the hand volume and indicates that the fall in the diastolic pressure was not the result of a peripheral relaxation of blood vessels.

The blood flow through the hand was determined by the Hewlett-Van Zwaluwenburg method in 12 subjects in 51 tests with negative results. The curve of the means determined for the amount of change in cubic centimeters above or below normal at regular intervals during and after the breathing of nitrogen has been plotted in figure 1. It shows no change and makes it clear that the changes in pulse rate and the arterial blood pressure that are induced by the acute anoxemia of nitrogen breathing do not cause an increase flow of blood through peripheral blood vessels.

SUMMARY

Curves of means showing the changes in pulse rate, arterial blood pressure, hand volume and volume of blood flow in the hand during a sudden and extreme anoxemia resulting from breathing pure nitrogen for about 82 seconds have been determined.

The heart rate began to accelerate within 10 seconds and thereafter increased smoothly and steadily throughout the nitrogen period. The return to the normal rate was made within about 35 seconds.

The mean response in the systolic arterial blood pressure was a gradual increase up to within about 10 seconds of the end of the nitrogen period, after which there was a fall. The return in the post-nitrogen period to about normal was made within 20 seconds.

The diastolic pressure response was a slight rise to about the middle of nitrogen period, then followed a progressive fall which continued not only through the remainder of the nitrogen period but for 20 seconds into the post-nitrogen period. After this the pressure began to recover and made the return to normal within 70 seconds after the end of the nitrogen period.

No changes of anoxemia occurred in the hand volume and the volume of blood flow through the hand.

We desire here to express our thanks to Major L. H. Bauer for assistance he has generously given.

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THE INFLUENCE OF THE HYDRION CONCENTRATION ON VASCULAR TONICITY

I. With Special Reference to Buffered Phosphate Perfusing Solutions, and the Specific Action of the Lactate Ion, in the Frog

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Many years ago Gaskell (1) demonstrated the possibility that vasoconstriction and vasodilatation might occur independently of nervous control through the effects of dilute acids and bases. He found that dilute acids tended to dilate the blood vessels of the frog, and that this action was specially marked with lactic acid. On the other hand, dilute solutions of sodium hydroxide (1:5000) caused a constriction of the vessels. It was later noted by Bayliss (2) that Ringer's solution saturated with carbon dioxide caused the leg vessels of a frog to relax, in comparison with the effect of normal Ringer's solution, and he suggested (3) that the carbon dioxide in this case acted as an acid. In experiments in which the whole frog was perfused, Hooker (4) found that calcium ions and oxygen increased the vascular tone, while sodium and potassium ions, carbon dioxide and urea decreased it. On the other hand, Pearce (5) concluded that neither dilute hydrochloric nor lactic acid had any vaso-dilating effect, but that they always produced vaso-constriction. Ishikawa (6) found that in frogs hydrochloric, lactic, and carbonic acids, sodium carbonate, sodium hydroxide, and even Ringer's solution itself caused a constriction of the blood vessels, but he admits that a vascular dilatation in a functioning organ under a reduction of blood alkali might depend upon the production of acid metabolites.

It occurred to us that the hydrion concentration of the perfusing solutions might be of importance in these effects, and moreover, that unless the perfusing solutions were buffered, the passage of body metabolites into the perfusing fluids might alter the results.

Experimental. With certain exceptions we used essentially the same procedures as were followed by Hooker (4). Instead of recording each drop perfused through the frog, we collected the perfused fluid in a tumbling trough, which emptied when filled to 1 cc., and recorded the moment of emptying, by electrical connections, upon the smoked drum. The use of a side tube near the point of entrance of the perfusing cannula to the vessels of the frog permitted us to wash out the perfusing tubes and to change from one solution to another more readily. We used frogs of approximately the same size and weight.

Buffered phosphate solutions prepared according to the directions of Clark and Lubs (7) were the perfusing solutions used, and in each case the actual hydrion concentration of the solution was determined electrometrically before using. Perfusion was begun with 0.7 per cent sodium chloride solution, and when it was flowing through clearly and at a constant rate, perfusion with the buffered solutions was started. Six series of three frogs each were followed. In series I we began with the strongest acid solution, followed with the strongest alkaline, and so alternated to the solution nearest neutrality. In series II we started with the strongest alkaline solution and alternated with the acid ones to the neutral point. In series III the perfusing solutions were followed consecutively from the most acid to the most alkaline, and in series IV, from the most alkaline to the most acid. Series V ran from neutrality to most basic, and series VI from neutrality to most acid. In every instance, when the record for any one perfusing solution had been obtained, the animal was perfused with 0.7 per cent saline until the rate of perfusion had nearly returned to normal for the saline, before the next buffered solution of the particular series was admitted. In the case of the more alkaline solutions this return to normal with saline was usually slow.

The results may be found in table 1. It will be noted that no matter what particular sequence was followed, the rate of perfusion was most rapid for those solutions having a pH of about 7.2. The rate of perfusion for solutions having a pH nearer 7.0 or 7.4 was not very different from that for a pH of 7.2, but was nevertheless slower. At a pH of about 7.6 marked constriction of the blood vessels seemed to take place, which increased as the alkalinity of the perfusing solutions rose. The constriction of the vessels occurring with solutions more acid than 7.0 was not as sudden nor as great as for the alkaline solutions with pH greater than 7.4. In all cases, the rate of perfusion was slower than that for 0.7 per cent saline.

TABLE 1

The effect of buffered phosphate perfusing solutions of known hydrion concentration on the rate of perfusion in frogs. Figures in seconds per cubic centimeter.

SERIES	PERFUSATE	0.7% NaCl	pH	pH	pH 6.29	pH 6.51	pH 6.70	pH 6.87	pH 7.09	pH 7.29	pH 7.46	pH 7.65	pH 7.92
I. Acid alternating with basic to neutral	Frog 1	19			69	44	35	38	32	30	35	83	173
	Frog 2	17			58	41	39	36	26	25	30	71	197
	Frog 3	17			73	51	44	39	33	29	37	64	207
Average................		18			67	45	39	38	30	28	34	73	192
	PERFUSATE	0.7% NaCl	pH	pH	pH 6.29	qH 6.51	pH 6.70	pH 6.87	pH 7.09	pH 7.29	pH 7.46	pH 7.65	pH 7.92
II. Alkaline alternating with acid to neutrality	Frog 4	26			67	56	48	41	35	35	42	146	226
	Frog 5	18			58	45	41	39	31	27	32	110	218
	Frog 5	20			66	51	46	38	33	31	36	121	196
Average................		21			64	51	45	39	33	31	37	126	213
	PERFUSATE	0.7% NaCl	pH	pH	pH 6.22	pH 6.46	pH 6.63	pH 6.78	pH 7.03	pH 7.21	pH 7.44	pH 7.58	pH 7.83
III. From most acid to most alkaline	Frog 7	16			69	35	38	32	25	22	25	78	181
	Frog 8	20			50	43	40	38	34	31	45	68	92
	Frog 9	18			55	44	41	33	28	30	41	72	108
Average................		18			58	41	40	34	29	28	37	74	123
	PERFUSATE	0.7% NaCl	pH 5.85	pH 6.03	pH 6.28	pH 6.44	pH 6.61	pH 6.82	pH 6.99	pH 7.22	pH 7.41	pH 7.63	pH 7.78
IV. From most alkaline to most acid	Frog 10	19	44	67	43	39	36	33	31	29	46	103	181
	Frog 11	21	86	78	66	44	38	35	30	30	54	118	198
	Frog 12	18	42	40	51	40	35	31	29	28	38	96	147
Average.........	Frog 12	19	57	61	53	41	36	33	30	29	46	105	175
	PERFUSATE	0.7% NaCl	pH	pH	pH	pH	pH	pH	pH 7.03	pH 7.24	pH 7.45	pH 7.61	pH 7.87
V. From neutral to most alkaline	Frog 13	15							20	18	19	46	103
	Frog 14	20							25	25	27	52	161
	Frog 15	18							21	20	23	57	121
Average................		18							22	21	23	52	128
	PERFUSATE	0.7% NaCl	pH 5.91	pH 6.08	pH 6.21	pH 6.38	pH 6.63	pH 6.79	pH 7.02	pH	pH	pH	pH
VI. From neutral to most acid	Frog 16	17	48	51	40	40	32	28	24				
	Frog 17	14	68	56	42	30	26	23	21				
	Frog 18	17	78	49	47	35	30	28	23				
Average................		16	65	52	43	35	29	26	23				

Specific action of the lactate ion. Buffered phosphate solutions were made as before, and 0.3 cc. of normal sodium lactate was added to every 200 cc. of each phosphate solution. The pH of the solutions was determined electrometrically before perfusion was started. We ran a series of five frogs, in which lactated buffered phosphate ("L")

TABLE 2

Comparison of the rates of perfusion of buffered phosphate solutions with lactated buffered phosphate solutions of approximately the same pH (0.3 cc. of normal sodium lactate per 200 cc. phosphate solution). Figures in seconds per cubic centimeter "P" = phosphate solution; "L" = lactated phosphate solution

	FROG NUMBER				
	1	2	3	4	5
0.7 per cent NaCl	24	20	19	20	21
"L" pH 6.23	64	58	60	55	61
"P" pH 6.26	76	67	72	61	70
"L" pH 6.70	45	44	46	42	48
"P" pH 6.72	48	50	49	47	53
"L" pH 7.16	32	31	30	29	33
"P" pH 7.12	35	33	31	31	35
"L" pH 7.30	28	26	27	26	30
"P" pH 7.42	43	38	35	34	39
"P" pH 7.48	29	30	30	29	35
"L" pH 7.72	34	36	33	34	38
"P" pH 7.61	75	68	58	61	86
"L" pH 7.88	60	55	47	51	53
"P" pH 7.86	121	116	101	113	133

solutions alternated with non-lactated buffered phosphate ("P"), solutions of approximately the same pH. From the data shown in table 2, it may be seen that in every instance the "L" solution perfused at a faster rate than the corresponding "P" solution. However, where the dilatation of the vessels was apparently near a maximum, as for solutions with a pH around 7.2, the differences in the rates of perfusion of the two types of solutions were slight. This difference was most marked on the alkaline side where, for example, an "L" solution of considerably higher pH value (pH 7.72) than a "P" solution (pH 7.42) still perfused at a faster rate. The general response of the

vascular tone to a sequence of lactated buffered phosphate solutions was the same as for normal buffered phosphate solutions, although at an accelerated rate (table 3) and without the marked constricting effect in the case of the more alkaline solutions.

Discussion. In perfusing frogs with Ringer's solutions containing gum arabic, it has recently been noted by Atzler and Lehmann (8) that both the hydrogen ion and the hydroxyl ion have a constricting action on the blood vessels as long as the concentration of these ions exceeds a certain limit, which is lower for the hydroxyl ion than for the hydrogen ion, and that the normal hydrion concentration of blood lies within the effective range of the hydroxyl ion. Later, these authors (9) showed that the extent of constriction depends not only on the hydrion concentration, but also on the degree of buffering. Thus,

TABLE 3

Effects of lactated buffered phosphate perfusing solutions on the caliber of blood vessels in frogs (0.3 cc. normal sodium lactate per 200 cc. phosphate solution). Figures in seconds per cubic centimeter

FROG NUMBER	0.7% NaCl	pH 5.56	pH 6.23	pH 6.70	pH 7.03	pH 7.16	pH 7.30	pH 7.48	qH 7.72	pH 7.88
1	19	78	63	47	30	27	27	28	36	63
2	22	70	65	43	34	33	33	34	39	58
3	21	66	58	41	29	26	26	28	35	54
Average...	21	71	62	44	31	29	29	30	37	58

they found that strongly buffered solutions were ineffective within a pH range from 5.65 to 7.6; a weakly buffered solution was inactive between pH 4.2 and 7.45, and unbuffered solutions had no effect between pH 2.9 and 9.35. It was concluded that frog's tissues have the power of so altering a perfusing solution of abnormal hydrion concentration that it approaches the hydrion concentration of blood, and that the less the solution is buffered, the more complete this alteration becomes. Fleisch (10) has also noted that the buffer value of perfusion fluids must be taken into account in discussing changes in vascular tonicity. In his experimentation he found that weak acids tended to cause a dilation, whereas stronger acids brought about a vaso-constriction.

Our results offer independent corroboration of the general findings of Atzler and Lehmann (8), (9). However, we wish to point out that the action of a well-buffered phosphate solution is effective within a

much narrower pH range than in the case of the solutions used by them. Indeed, this range is so small in our series that it forces us to believe that so far as a well-buffered solution is concerned, the maximum dilatation of the blood vessels occurs within the limits of the hydrion concentration of normal blood, with indications that the vessels constrict more sharply for alkaline variations than for acid. We were unable to obtain blood in sufficient amounts from frogs to determine directly the pH of frog's blood, at least on samples which we could be certain had not been exposed to air. Our indications were that the pH of frog's blood is slightly lower than the figure usually accepted for mammalian blood (7.4).

From our observations it may be inferred that in the intact body greater facility is given for the removal of acid waste products from the tissues than for the entrance of alkaline blood into them. The response of the blood vessels to the hydrion concentration of the fluids passing through them appears to be protective in both directions. Blood is a relatively strong buffered solution, and it would seem therefore that slight variations in its hydrion concentration would affect the caliber of the vessels through which it flowed.

The protective response of the blood vessels to the fluids passing through them is emphasized by their reaction to the lactate ion. Here, even though the pH of the perfusing fluid may be considerably more on the alkaline side than normal blood, the vessels still fail to constrict. The importance of this specific action of the lactate ion to the organism is apparent when muscular effort is considered: carbon dioxide is usually blown off during sudden and severe muscular work, and the blood becomes alkaline; if the vessels responded to this alkalinity by constriction, the impairment of circulation would be serious, especially locally, but the passage into the vessels of even minute amounts of the lactate ions, which might perhaps escape oxidation in the muscle, apparently would be sufficient to hold them open and to maintain effective circulation.

It has been suggested by Macleod (11) that the excess of lactic acid in the blood in anoxemia may perform the function of assisting in the neutralization of the relatively increased base which results from the blowing off of carbon dioxide from the blood, this being dependent upon stimulation of the respiratory center by the oxygen deficiency. We offer the suggestion that in addition to this function, the lactic acid may assist materially in dilating the blood vessels and maintaining adequate circulation when, in the condition under discussion, the

vessels would otherwise be expected to constrict, due to the increased alkalinity of the blood.

With regard to the local effect of acid metabolites, the conclusions of Fleisch (12) are of interest. By means of a method for estimating the blood supply to different organs at work and at rest, he found that the acid products formed during tissue activity possess a vasodilating property, and that the blood supply is increased in proportion to the quantity of acid metabolites produced. He concluded that the intensity of the dilatation of the vessels is a function of the hydrion concentration, and that every organ and every segment of tissue receives just the amount of blood it needs for the work it is doing at the moment.

Whether our results are to be interpreted from the point of view of the vascular tonicity as a whole, or of a certain portion of the vascular system, such as the capillary bed, we are not prepared definitely to decide. The direct observation of the capillaries and smaller vessels of a perfused frog is not satisfactory, although it seemed that constriction and dilatation could be observed in accordance with the figures obtained for the different rates of perfusion. Gaskell (1) and Atzler and Lehmann (8), (9) report microscopic observations of the capillaries which support their more quantitative findings. Hooker (13) states that the capillary bed is responsive to both chemical and nervous influences, and that the chemical factors, so far as they have been studied, mediate dilatation of the capillaries and venules, while nerve stimulation mediates constriction of these vessels. It has been stated (14) that we may believe that the chemical regulation is usually local in character, producing changes in accordance with the passing needs of particular tissues, and that only in special or pathologic conditions does the reaction extend to the body as a whole, and that in accordance with this view we might regard the nervous control as a force tending to restrict the capillary beds over the body as a whole, thus maintaining a tone to be played upon by chemical factors. Most of our animals were pithed, but in some, movements of the fore-legs and hind-legs indicated that the reflex centers of these parts of the body at least had not been destroyed. There was no apparent difference in the rate of perfusion, or in the changes in the rate of perfusion with different solutions, between such animals and those in which pithing had apparently completely destroyed the spinal cord. We are inclined therefore to feel that the hydrion concentration of the fluid

contained in the blood vessels is also an important factor in vascular tonicity, and that it acts not only locally, but generally throughout the body. Indications lead to the belief that maximum dilatation occurs with a pH slightly more acid than the pH of normal blood, but that so far as chemical substances which alter the acid base equilibrium of blood are concerned, they produce constriction of the blood vessels, if the change is beyond the pH range of say 7.1 and 7.4. On the other hand, certain chemical substances such as histamine (15) seem to be specific capillary poisons, effecting a dilatation beyond any secured by probable changes in the pH.

The rate of diffusion of different ions is undoubtedly of significance in the effects under discussion, and investigation along this line is under way. In this connection the isotonicity of the perfusing fluids may influence the results. We did not accurately control the isotonicity of our perfusing solutions, but made rough tests on them with respect to hemolysis of dog's red blood corpuscles in vitro. We found all the buffered phosphate solutions with pH above 6.8 of sufficient salt content to keep hemolysis from taking place. To those below pH 6.8 we added sodium chloride in adequate quantities to prevent hemolysis.

SUMMARY

Perfusion of frogs with buffered phosphate solutions of known hydrion concentration shows that maximum dilatation of the blood vessels occurs for pH values in the neighborhood of 7.2. Constriction takes place sharply when the perfusing fluid has a pH greater than 7.4, and while the vessels constrict for pH values less than 7.2, the response is not so sudden, nor so marked.

The addition of very small amounts of lactate ions to buffered phosphate solutions greatly diminishes the constricting effect of the solutions upon perfusion. The influence of the lactate ion is more marked on the alkaline side of blood reaction, where a lactated buffered phosphate solution will cause considerable dilatation in comparison with a non-lactated buffered phosphate solution of even higher pH.

The protective value of these vascular responses to changes in the hydrion concentration of buffered perfusion solutions, and of the specific effect of the lactate ion, is pointed out.

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EFFECTS FOLLOWING THE INTESTINAL ADMINISTRATION OF (ILETIN) INSULIN

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Rapid intestinal absorption of an undigested protein substance was pointed out by one of us (C. A. M.) in a paper read before the American Physiological Society, December 28, 1922, at Toronto. In this paper (1) were described the effects on the blood-clotting time of tissue fibrinogen (the blood coagulant of the tissues) placed in the small intestines of animals, or taken orally under special conditions by man. Absorption of the unchanged protein was found to be remarkably rapid, evidence of its presence in the blood being noted $2\frac{1}{2}$ minutes after swallowing the protein solution. Rees and Whitehead (2) have also recently reported an instance of very rapid absorption of pituitrin from the duodenum. They demonstrated that the uterine stimulating action of the pituitrin is observed within 50 seconds after placing the drug in the duodenum.

Effects following intestinal administration, if absorption takes place, are more rapid in onset than those following subcutaneous, intramuscular or intraperitoneal injections. Effects of the former appear in less than 3 minutes, those of the latter appear in 20 to 60 minutes. On the other hand, when the desired effects are once produced they are shorter in duration by the enteral route than by the other routes. Thus, although the results obtained by enteral administration of active drugs are very prompt, they are fleeting in their action.

The usefulness of insulin in the treatment of diabetes mellitus has led many investigators in this field to seek a more satisfactory method of administration than that offered by subcutaneous injection. This latter method is always associated with a moderate amount of discomfort. Owing to the evanescent nature of the action of insulin, the injections must be repeated twice or thrice daily, depending on the severity of the case, over long periods of time. This is rather trying for

the patient, and in some cases the individual develops such a phobia for the needle that treatment is necessarily discontinued.

Rectal injections in man were attempted, using large quantities of insulin, but no effect was noted. Banting and Best (3) also found that rectal administration was without effect in dogs, while slight results could be sometimes obtained when insulin was inserted through a stomach tube. These investigators concluded that the subcutaneous and intravenous injections gave the most dependable results.

Intranasal insufflation was tried after the method of Blumgart (4), but without success.

In view of the results obtained by one of us (C. A. M.) with tissue fibrinogen, the authors decided to investigate the intestinal route of administration more closely. Sutter, Gibbs and Murlin (5) reported a reduction in hyperglycemia and glycosuria following the administration of their pancreatic extracts directly into the duodenum of patients through an Einhorn tube. Concentrated extracts given by mouth were useless, except when used in enormous quantities and accompanied by sufficient alkali to neutralize the HCl of the stomach contents.

Methods. Well-fed adult rabbits were used for the experiments. Later, after rabbit experimentation was carefully controlled, several experiments were made on the human. Iletin (insulin), H-10[1] supplied by Eli Lilly & Company, was used in these experiments. Blood for sugar determinations was obtained from the marginal ear veins and examined within ½ hour after withdrawal. The blood sugar method of Folin and Wu (6) was used throughout these experiments.

Control rabbits to show the activity of the insulin used:

Rabbit 1. Weight 2000 grams

1:40 p.m. Blood sugar 0.13 per cent
1:56 p.m. 1 cc. (10 units) of iletin given subcutaneously
3:30 p.m. Blood sugar 0.056 per cent

Rabbit 2. Weight 2000 grams

2:00 p.m. Blood sugar 0.145 per cent
2:10 p.m. 1 cc. (H-10) iletin given subcutaneously
4:30 p.m. Blood sugar 0.057 per cent

There was a marked diminution in the blood sugar. No convulsions or other symptoms of hypoglycemia developed in either of these rabbits during 5 hours of observation. A third rabbit, however, went into convulsions one hour after receiving 2 cc. (20 units) of the insulin subcutaneously and was restored by 10 grams of glucose administered under the skin. Blood sugar determinations were not made on this rabbit.

[1] H-10 refers to the preparation of iletin which contains 10 units to the cubic centimeter.

A series of experiments was now performed to show the effects of insulin placed directly in a loop of intestine. Blood was drawn for sugar determination. The animal was then anesthetized with ether (open cone) and the abdomen opened as aseptically as possible, by an incision $1\frac{1}{2}$ inches long. A loop of small intestine was carefully and tenderly brought to the incision and iletin (insulin) was therein injected with needle and syringe. The loop of gut, which ballooned out slightly when the injection was made, was carefully replaced in the abdominal cavity under loops of large intestine which seemed to be superficially situated beneath the peritoneum. The abdominal incision was then closed by means of 3 black silk sutures, the edges of the wound being carefully coapted. The duration of ether administration averaged 10 minutes.

Control experiment showing the hyperglycemic effect of etherization:

Rabbit 6. Weight 2200 grams

1:40 p.m. Blood sugar 0.159 per cent

2:25 p.m. Animal etherized, abdominal cavity opened, small loop of gut brought to surface and then replaced. *No iletin (insulin) given.* Incision closed as described above

Animal recuperated

3:10 p.m. Blood sugar 0.323 per cent

3:40 p.m. Blood sugar 0.247 per cent

In the above experiment there was an increase in the blood sugar concentration amounting to over 100 per cent in 45 minutes following ether anesthesia. In 1 hour and 15 minutes the blood sugar showed a definite decline.

Rabbit 4. Weight 2500 grams

2:15 p.m. Blood sugar 0.13 per cent

3:00 p.m. Animal etherized, abdomen opened and *4 cc. of iletin (H-10)* were injected into a loop of small intestine at 3:06 p.m.

4:25 p.m. Blood sugar 0.092 per cent

Thus in 1 hour and 20 minutes after etherization plus insulin, the blood sugar was below the normal for this animal when one would expect an increase of over 50 per cent.

Rabbit 5. Weight 2400 grams

1:35 p.m. Blood sugar 0.20 per cent

2:07 p.m. Animal etherized, etc., as above and 6 cc. of iletin (H-10) were injected into intestinal loop

2:37 p.m. Blood sugar 0.151 per cent

3:07 p.m. Blood sugar 0.182 per cent

4:07 p.m. Blood sugar 0.182 per cent

Results shown here are the same as those obtained with rabbit 4. A definite lowering of the blood sugar is noted within ½ hour. Within 1 hour the blood sugar has almost returned to the normal level.

It was decided to perform an experiment similar to the above, taking blood sugars at more frequent intervals to determine how soon the blood sugar lowering effect of insulin could be detected after etherization.

Rabbit 7. Weight 2500 grams

1:30 p.m.	Blood sugar 0.174 per cent
1:43 p.m.	Animal etherized and operated
1:47 p.m.	5 cc. of iletin (H-10) injected into lumen of small gut
1:55 p.m.	Blood sugar 0.256 per cent
2:08 p.m.	Blood sugar 0.247 per cent
2:25 p.m.	Blood sugar 0.204 per cent
3:15 p.m.	Blood sugar 0.247 per cent
4:40 p.m.	Blood sugar 0.244 per cent

This experiment shows that insulin almost immediately curbs the hyperglycemia following ether administration, although the maximum effect in this case was not as evident as that seen in the previous experiments.

A similar experiment was performed on a dog with almost identical results.

Dog. Weight 14 kilos

This animal was used for another experiment. Ether anesthesia had lasted over a period of 3 hours and adrenalin had been given ½ hour before the first specimen of blood was drawn for sugar determination.

6:00 p.m.	Blood sugar 0.520 per cent
6:01 p.m.	5 cc. of iletin (H-10) were injected into the lumen of small intestine
6:15 p.m.	Blood sugar 0.393 per cent
6:30 p.m.	Blood sugar 0.422 per cent

The effects of enterally administered iletin (insulin) appeared so promptly (15 to 30 minutes), that one was led to inquire whether this method was even more rapid in its resultant action than subcutaneous or even intravenous administration. Two more experiments were therefore performed, in one case iletin (insulin) was given subcutaneously, in the other case intravenously, and blood sugars were done at frequent intervals.

Rabbit 9. Weight 2300 grams

1:25 p.m.	Blood sugar 0.256 per cent
2:06 p.m.	25 units of iletin (H-10) given subcutaneously, in two places

2:21 p.m. Blood sugar 0.175 per cent
2:36 p.m. Blood sugar 0.100 per cent
3:06 p.m. Blood sugar 0.093 per cent
4:00 p.m. Animal showed evidences of weakness and prostration
4:30 p.m. Convulsions and coma
4:42 p.m. 20 cc. of 50 per cent glucose given subcutaneously
4:46 p.m. Recovery

Rabbit 8. Weight 2400 grams
1:15 p.m. Blood sugar 0.208 per cent
1:46 p.m. 25 units iletin (H-10) intravenously (ear vein). No excitement or anesthesia
2:01 p.m. Blood sugar 0.163 per cent
2:16 p.m. Blood sugar 0.111 per cent
2:46 p.m. Blood sugar 0.105 per cent
4:00 p.m. Weakness and prostration
4:30 p.m. Partially recovered but still weak and listless
4:35 p.m. 20 cc. of 50 per cent glucose given subcutaneously to avoid coma and convulsions

Thus it will be seen that subcutaneous and intravenous injections of insulin are followed by equally as prompt lowering of the blood sugar as is observed after enteral administration.

The results of all these experiments are represented graphically in chart 1.

Having demonstrated definite intestinal absorption of iletin (insulin) in rabbits and dogs, experiments on normal and diabetic men seemed warranted. Iletin was administered to one of the authors (C. A. M.) after a method devised by himself (1) as follows:

(C. A. M.) Normal adult.
9:50 a.m. Blood sugar (no breakfast) 0.089 per cent
10:00 a.m. 2 cc. of iletin (H-10) were given orally with a glass of ice water
10:45 a.m. Blood sugar 0.095 per cent

The experiment was repeated using a larger quantity of iletin.
11:32 a.m. Blood sugar (no breakfast) 0.109 per cent
11:34 a.m. 5 cc. of iletin taken as above
12:05 p.m. Blood sugar 0.106 per cent

To be sure that the material was reaching the intestine rapidly, 1 cc. of 1.5 per cent solution of tissue fibrinogen was given with the insulin and the clotting time of the blood observed at the same time. One can see that the clotting time is promptly shortened, indicating ready absorption of the coagulant. The ice water could also be felt as it entered the intestine and yet there was no reduction of the blood sugar following ingestion of 4 cc. of iletin.

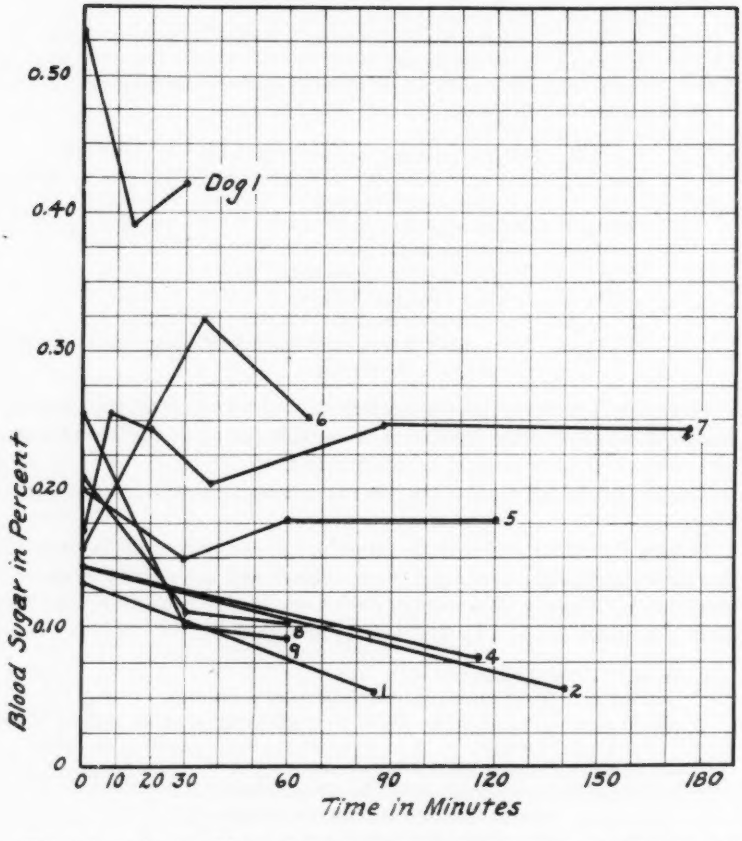

Chart 1. *1, 2, 9.* Effect of subcutaneous administration of iletin on the blood sugar of normal rabbits.

4, 5, 7. Effect of the intestinal administration of iletin following ether anesthesia.

6. Ether hyperglycemia plus abdominal operation. Control rabbit.

8. Effect of intravenous injection of iletin on the blood sugar of a normal rabbit.

C. A. M.

11:20 a.m.	Blood clotting time 2 minutes 45 seconds
11:35 a.m	Blood sugar 0.083 per cent
11:38 a.m	4 cc. of iletin given as above with 1 cc. of tissue fibrinogen
11:43 a.m	Blood clotting time 2 minutes and 10 seconds
11:48 a.m.	Blood clotting time 2 minutes and 30 seconds
12:38 p.m.	Blood sugar 0.084 per cent

The same experiment was tried on a diabetic patient over a period of 24 hours as follows:

J. H. Severe diabetic. Body weight 102 pounds.
12–19–22. 8:20 p.m. Blood sugar = 0.226 per cent
Glycosuria for the 24 hour period = 27.0 grams
12–20–22. Insulin was administered as above:
50 units (5 cc.) before breakfast
75 units (7½ cc.) before dinner
200 units (20 cc.) before supper
8:20 p.m. Blood sugar = 0.238 per cent
Glycosuria for the 24-hour period = 31.7 grams
12–21–22. Glycosuria for this 24 hour period = 24.0 grams

Thus, the administration of 325 units by mouth during a 24-hour period had practically no effect on the blood and urinary glucose. This amount was just eight times the quantity necessary to keep the patient sugar-free when the insulin was administered subcutaneously.

The experiment of Sutter, Gibbs and Murlin was repeated on a diabetic patient, using iletin.

Wm. R. Moderately severe diabetic. Weight 116 pounds.

7:30 a.m	Breakfast
10:30 a.m	Rehfuss tube passed. There was considerable gagging, so throat was painted with 5 per cent cocaine solution
12:00 noon	Fluoroscopic examination revealed bulb of tube in the second portion of duodenum (Dr. Little)
12:05 p.m.	Blood sugar 0.241 per cent
12:12 p.m.	10 cc. of iletin (insulin) were given through the tube followed by 30 cc. of water and 30 cc. of air
12:40 p.m.	Blood sugar 0.33 per cent
1:23 p.m.	Blood sugar 0.317 per cent
4:20 p.m.	Blood sugar 0.300 per cent

Thus one can see that in man, insulin placed in the small intestine, even in large quantities, has no influence on the blood sugar. In the last case, for reasons unexplained, there was a definite rise in the blood sugar.

DISCUSSION AND CONCLUSIONS

A survey of the experiments and careful scrutiny of chart 1 reveal the following facts:

1. Insulin placed in the small intestine of rabbits and dogs causes a very prompt lowering of the blood sugar level in ether hyperglycemia.
2. Although the effect of iletin by the intestinal route is prompt, its action is not sustained, lasting only an hour.
3. In 1 and 2 one sees again evidence of fairly rapid absorption from the small intestine of a substance that is destroyed by digestion.
4. To produce the desired effect by intestinal administration huge doses of iletin are required.
5. Subcutaneous and intravenous administration of small quantities of iletin give prompt results as well as sustained reactions, lasting over a period of several hours.
6. In man, the blood sugar lowering effect of iletin (insulin) was not noted after good sized doses were administered by the intestinal route.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

VII. The Effect of Insulin on the Blood Sugar Following Total and Partial Removal of the Liver

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At the request of Macleod and Banting, we have investigated the effect of insulin on the blood sugar following total and partial removal of the liver. Our previous studies (3), (4) on the effect of removal of the liver had demonstrated that *a*, a decrease in blood sugar always occurs; *b*, the hypoglycemia is always accompanied by a characteristic group of symptoms; and *c*, the administration of glucose abolishes the symptoms and temporarily restores the animal to normal. The discoverer and investigators of insulin have found (1) that its administration is followed by hypoglycemia, the development of a characteristic group of symptoms, and that glucose is effective in relieving the symptoms. There is, thus, a very close similarity between the effect of removal of the liver and the administration of insulin.

The problem was the determination, if possible, of whether the liver is responsible in whole, or in part, for the hypoglycemia following the administration of insulin. We know, as the result of many experiments, the form of the curve of blood sugar following removal of the liver. A control curve of blood sugar could be obtained by the administration of insulin before removal, and if the amount of insulin given were sufficient, a much more precipitate drop in the curve of blood sugar would be produced by the insulin than by removal of the liver. If the administration of the same amount of insulin after removal did not alter the blood sugar curve from that usually occurring after removal, it would be evidence that the liver is of importance in the hypoglycemic action of insulin. On the other hand, if the curve of blood sugar following the administration of insulin was not affected by removal, it would show that the presence of the liver is not necessary for the hypoglycemic action of insulin.

The foregoing series of experiments was performed and the latter alternative, that total removal of the liver did not affect the hypoglycemic action of large doses of insulin, was found to be true. However, the experiments did not prove, since they were negative, that the liver did not play some part in the hypoglycemic action of insulin. It can readily be seen that if the action of insulin in reducing the blood sugar were a storage phenomenon, involving both the muscles and liver, it would be possible with the large amount of insulin administered for the muscles to take enough sugar from the blood to produce the hypoglycemia obtained. In this event the liver, in all probability, would also be an active participant, but the amount of muscle tissue being so large and being stimulated by the insulin, the small amount of available glucose could be taken up so rapidly that any effect the loss of the liver might have would be masked. In order to investigate this point, another series of experiments was performed in which small amounts of insulin were administered before and after partial removal of the liver. If the liver is partially responsible for the hypoglycemic action of insulin, it would seem that if an amount of insulin were employed which would produce only a slight decrease in blood sugar, the curves of blood sugar before and after removal of more than half of the liver would be different. An amount of insulin which would produce a measurable hypoglycemia with the liver intact should produce little or no reaction with a large portion of the liver removed, if the liver was of much importance in this hypoglycemic action of insulin. It would seem necessary to leave a portion of the liver intact in order not to have hypoglycemia due to loss of liver function, as well as to determine the part the liver might play in the recovery from the insulin.

Method of experimentation. Dogs were used in all experiments; the operative procedures were carried out under ether anesthesia and sterile technic. We have published our method for total removal of the liver in detail (2), and a note (5) on partial removal of the liver. These need not be repeated here. However it should be noted that the animals employed in the series of experiments in which the liver was partially removed, had been subjected to an Eck fistula operation several weeks before, and the functional capacity of the liver was therefore greatly reduced. The insulin used was obtained through the courtesy of Macleod and Banting. The animals had been prepared for the operations several months before. In three of the hepatectomized animals a control curve of blood sugar following the injection of insulin was obtained before the operation; in one experiment the

insulin was not given until afterward; in all experiments in which partial hepatectomy was performed, a control injection was given. All specimens of blood were obtained from the jugular veins, and all injections were made into these veins. The blood sugar estimations were made by the Benedict modification of the Lewis-Benedict method, the Folin and Wu method, and the Shaffer and Hartman method. There was no essential difference in the results obtained by the three methods.

The routine procedure, in the experiments in which insulin was injected twice, was as follows: The animal was fasted for from sixteen to eighteen hours. Two or more blood sugar estimations were made at half-hour intervals to obtain the normal blood sugar level. Insulin was then injected intravenously. In order to show whether there was a difference in the use of insulin before and after removal of the liver, it was necessary to obtain a more precipitate curve following the administration of insulin than occurs following removal of the liver. Accordingly a large dose of insulin, one unit for each kilogram of body weight, was given in this series of experiments. In the series in which partial hepatectomy was performed, an amount of insulin which would just produce a measurable hypoglycemia was employed; in this series, 0.125 to 0.25 unit for each kilogram of body weight was given. Blood sugar estimations were made at frequent intervals afterward, and the condition of the animal was noted. In those instances in which symptoms developed, and it was necessary, glucose was administered and the estimations of blood sugar made until the animal had been apparently normal for two or more hours. After a variable period, from one to four days after the experiment, the animal was again fasted, a normal blood sugar estimation made, and the liver totally or partially removed, with our usual technic. Blood sugar estimations were made at short intervals following the operation. When the animal had recovered from the immediate effects of the anesthetic and operative procedures usually within an hour, the blood sugar was again estimated and the same amount of insulin injected as in the experiment before the removal of the liver. Blood sugar estimations were made at practically the same intervals as in the control experiment. The condition was again carefully noted and, when necessary, glucose was injected. The experiment was thus maintained for several hours after operation. In the experiment in which insulin was not administered before the liver was totally removed, only the procedure following the removal was employed. This experiment was performed in order to determine

whether the insulin administered before removal influenced the blood sugar and action of insulin after removal. In order to make the blood sugar curves following the administration of insulin before and after operation more comparable, in one experiment anesthesia was induced before the control injection of insulin for the same length of time as was necessary for the partial removal of the liver.

Results of the experiments. The results of the experiments to determine whether or not the hypoglycemic action of insulin is necessarily dependent on the liver, are very definite. The injection of large doses of insulin in dogs before total removal of the liver produced marked hypoglycemia; the decrease in blood sugar was much more rapid than occurs after removal. In two of the animals symptoms developed which were, in the main, identical with those occurring with the hypoglycemia following removal, and the injection of glucose produced the same immediate restoration to normal. Slight symptoms only followed the injection of insulin in one animal and, although the blood sugar decreased to a very low level, the animal recovered without the administration of glucose. The injection of the smaller dose of insulin in the animals in which the liver was to be partially removed, produced definite hypoglycemia, but no symptoms.

The administration of insulin after both total and partial hepatectomy produced the same precipitate decrease in blood sugar as with the liver intact. It should again be emphasized that the hypoglycemia occurred much more quickly, and the symptoms, when they were present, were the same as if insulin had not been injected and only hepatectomy performed. The intravenous injection of glucose in the experiments following total hepatectomy produced the same quick response but whereas, when the liver was intact, the blood sugar level would gradually be restored and then maintained at normal, when the liver had been removed, the hypoglycemia and the associated symptoms recurred, making repeated injections necessary. The length of time a given amount of glucose maintained the animal following the injection of insulin after removal of the liver was variable. The average animal was maintained one hour by 0.25 gram of glucose for each kilogram of body weight. In one experiment in which insulin was injected, twice the amount was necessary; in another, considerably less was necessary.

Discussion. Although only four experiments were performed in which the liver was totally removed, and three in which it was partially removed, one of which was complicated with hemorrhage, the results with regard to hypoglycemia following the administration of insulin

before and after total and partial hepatectomy seem so definite that further experiments of this character did not appear necessary. The symptoms in the dog, when they are present, which are associated with the hypoglycemia of insulin are almost identical with those associated with the hypoglycemia following total removal of the liver. The flaccidity noted after the liver has been removed is not so noticeable after the administration of insulin, but the excitatory phenomena seem to be more marked after the latter. The blood sugar curve is not so regular after the administration of insulin as after removal of the liver. A dog may appear normal with hypoglycemia following insulin, whereas the symptoms would be profound if the same degree of hypoglycemia had followed removal of the liver.

The results of the experiments clearly prove that the presence of the liver is not necessary for the hypoglycemic action of insulin. On the other hand, they also prove that the presence of the liver is necessary for the permanent recovery of the blood sugar level. These results do not necessarily mean that the liver may not play a part in the production of the hypoglycemia of insulin. The fact that the liver is essential for the restoration of the normal blood sugar level proves that this organ is affected either directly or indirectly by the insulin.

It is not our purpose to attempt an explanation of the action of insulin. However, the blood sugar curve following the administration of insulin appears as a very precipitate curve after the liver has been removed. This might indicate that the hypoglycemic action of insulin may be at least partially produced, not only by inhibiting the functions of the liver in the maintenance of the normal level of the blood sugar, but by inhibiting whatever part the muscles might play in this function. The experiments definitely show that the liver is necessary for the permanent recovery and maintenance of the blood sugar level after insulin but do not definitely prove that the liver may not be an active participant in the hypoglycemic action of insulin, although certainly not a necessary participant. Unfortunately, in the series of experiments in which the liver was totally removed, for some unknown reason, the amount of glucose necessary to maintain the animal in a normal condition after operation was so irregular as to be of no value in determining whether or not the injected glucose disappeared more rapidly following insulin when the liver was intact as compared to the disappearance when the liver had been removed. This should be studied further. It should be noted that the characteristic symptoms which we have described as accompanying the hypoglycemia following

total removal of the liver and which the investigators of insulin have described following the injection of the substance are practically identical and are undoubtedly due to the hypoglycemia. It should also be noted that the hypoglycemia following hepatectomy is not due to the action of insulin, because in another study[6] we have shown that it occurs when both the pancreas and the liver are removed.

SUMMARY

Blood sugar estimations were made following the injection of insulin, before and after total and partial removal of the liver. The symptoms associated with the hypoglycemia following the administration of insulin do not differ essentially from the symptoms associated with the hypoglycemia following removal of the liver, and the action of glucose seems to be identical in the two conditions. However, the hypoglycemia following the administration of insulin may not always be associated with symptoms, while that following removal of the liver invariably produces symptoms. The effect of large doses of insulin in producing hypoglycemia is not changed by total removal of the liver, nor is the hypoglycemia action of small doses of insulin modified by the partial removal of the liver (the removal of approximately 60 per cent of the liver of an animal in which an Eck fistula had been performed a considerable time before). On the other hand, the liver is absolutely necessary for the permanent recovery of the blood sugar level. When the liver is totally removed, the blood sugar level cannot be maintained normally. When the liver is partially removed the return to normal following insulin is greatly retarded. These experiments prove that the liver is certainly not necessary for the hypoglycemic action of insulin, although they do not show that it is not directly or indirectly involved in such an action. The experiments also definitely prove that the liver is necessary for permanent recovery of the blood sugar level.

Protocol 1. Dog F376. Experiment 599. Male, adult mongrel dog, weighing 10.8 kgm. A reverse Eck fistula was made May 31, 1922. August 11, 1922, the dog weighed 10.9 kgm. The portal vein was ligated. February 19, 1923, the animal was in excellent condition, and the following experiment was performed. After having fasted 16 hours, the dog weighed 10.3 kgm. At 8:30 a.m. the first specimen of the blood was taken, and the blood sugar was found to be 0.096 per cent; at 9:00 it was 0.105, and at 9:30, 0.105. At this time one unit of insulin for each kilogram of body weight, or a total of 10.3 of the H/10 insulin, no. 729748, was injected intravenously. No immediate effect was noted. At 10:00 the fourth specimen of the blood was taken; the blood sugar was 0.043 per cent; at 10:30 it was 0.052 per cent. At this time the animal began to show the first signs of

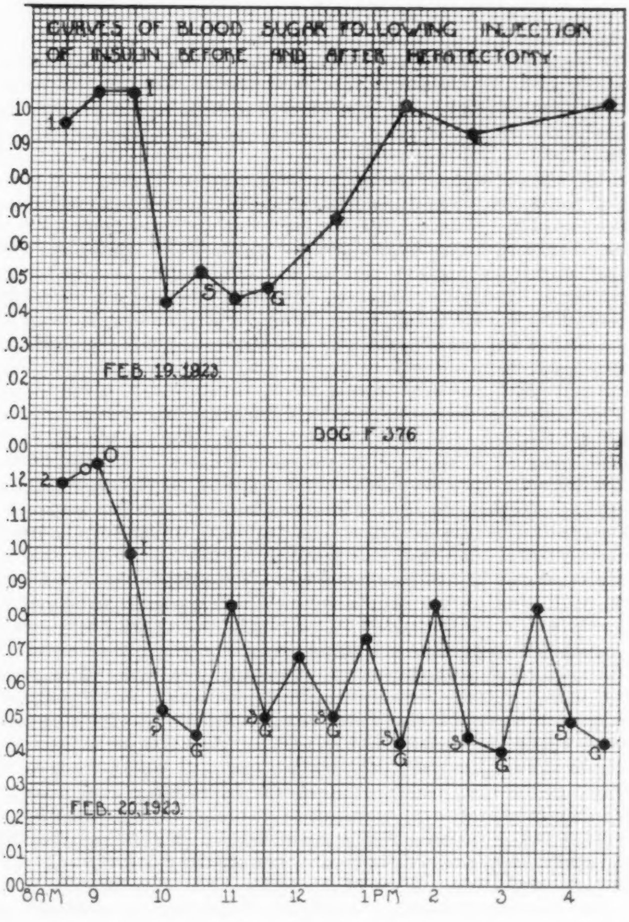

Fig. 1. *I*, injection of insulin; *O*, removal of liver; *S*, symptoms present; *G*, injection of glucose.

Curve *1* shows the effect of intravenous injection of 1 unit for each kilogram of body weight of insulin on blood sugar in an animal which had been prepared for removal of the liver by performing a reverse Eck fistula, and ligating the portal vein. Curve *2* shows the effect of injection of the same amount of insulin after total removal of the liver. Note that the same precipitate decrease in blood sugar occurs after the administration of insulin in the hepatectomized animal as when the liver was intact. But in the latter instance (curve *1*) the blood sugar level is restored and maintained at normal following the injection of glucose, but when the liver is removed (curve *2*) the permanent recovery of the blood sugar level is impossible (protocol 1).

muscular weakness. When put on the floor he walked, but his hind quarters swayed. At 11:00 the symptoms were more marked, and the blood sugar had dropped to 0.044 per cent. The symptoms progressed rapidly, so that at 11:30 the animal was perfectly flaccid, and in the identical condition noted in animals from which the liver had been totally removed. At this time the blood sugar was found to be 0.047 per cent. Five-tenths of a gram for each kilogram of glucose, or a total of 10.3 cc. of a 50 per cent solution, was injected intravenously. The animal immediately recovered; the recovery was just the same as following the injection of glucose in the hepatectomized animal. At 12:30 p.m. the blood sugar was 0.069 per cent; at 1:30, 0.101 per cent; at 2:30, 0.093 per cent, and at 4:00, 0.101 per cent. The animal remained in perfectly normal condition after the injection of glucose. He was next given a meal of 50 grams of glucose, 400 cc. of milk, and a small amount of bread, put back in the cage, and then fasted for the next experiment which was made on the following day.

February 20, 1923, the dog weighed 10.8 kgm. At 8:30 a.m. the blood sugar was 0.119 per cent. Dog was etherized at 8:35 and the liver removed at 8:53, the circulation of the liver being shut off at 8:45. The ether was withdrawn at 9:05. The animal weighed 10.3 kgm. after the operation, which was relatively easy. An estimation of the amount of blood lost was 35 cc. The liver was small, weighing 215 grams. At 9:00 the blood sugar was found to be 0.125 per cent. The animal was taken from the operating room, artificial heat applied, and it began to recover very nicely. At 9:30 it had recovered from the anesthetic so that it could walk around, respond to call, and so forth. At this time the blood sugar was 0.097 per cent. One unit of insulin for each kilogram of body weight was injected, and as the animal weighed 10.3 kgm., the relationship was exactly the same as on the preceding day. At 10:00 the blood sugar was found to be 0.052 per cent. The animal showed some signs of muscular weakness, which progressed rapidly so that at 10:30 the animal was perfectly flaccid and could not stand. The blood sugar was then 0.045 per cent. It was necessary to give glucose at this time, and 0.5 gram for each kilogram of body weight was injected intravenously. At 11:00 the blood sugar was 0.083 per cent, and at 11:30, 0.050 per cent. The animal had again become perfectly flaccid and glucose was required; accordingly the same amount was again injected. At 12:00 the blood sugar was 0.068, and at 12:30 p.m., 0.050. The animal was again flaccid, and glucose was injected. At 1:00 the blood sugar was 0.073 per cent, and at 1:30, 0.042; the animal had become flaccid, and the same amount of glucose was injected as before. At 2:00 the blood sugar was 0.084 per cent. At 2:30 the animal was again flaccid, and the blood sugar was 0.044 per cent. However, the animal was allowed to remain in this flaccid state, which grew progressively worse, and at 3:00 the blood sugar had dropped to 0.041 per cent, and glucose was injected as before. At 3:30 the blood sugar was 0.082 per cent, but at 4:00 had dropped to 0.049 per cent, and the animal was again flaccid. However, no more glucose was administered. At 4:30 the blood sugar was 0.041 per cent, and at 5:00, 0.038 per cent. At this time the animal showed marked twitchings. At 5:30 the blood sugar was 0.037 per cent. The animal was then bled to death under ether. Necropsy was performed immediately, and nothing was found which would invalidate the results of the experiment.

Protocol 2. Dog F345. Experiment 442. Male, adult mongrel. Weight 13.9 kgm. April 21, 1922, a reverse Eck fistula was made. May 16, 1922, the dog weighed 14.0 kgm., and the portal vein was ligated. February 22, 1923, the animal was still in excellent condition, and the following experiment was performed.

After having fasted 16 hours, the dog weighed 15.5 kgm. At 8:30 a.m. the first specimen of the blood was taken and the blood sugar was found to be 0.101 per cent. The animal was etherized at 8:30; the first incision was made at 8:40 and the liver removed at 8:45. Ether was withdrawn at 9:00. Very little blood, less than 50 cc., was lost at the time of operation. The animal weighed 14.9 kgm. after operation. The liver weighed 365 grams. At 9:00 the blood sugar was found to be 0.10 per cent; at 9:15, 0.103 per cent; at 9:30, 0.096 per cent, and at 9:45, 0.087 per cent. The animal had by this time recovered from the immediate effects of operation. One unit of insulin for each kilogram of body weight, or a total of 1.5 cc. of the H/10, no. 729748, was injected. At 10:00 the blood sugar was 0.08 per cent, and at 10:15, 0.048 per cent. Well-marked symptoms were present, such as muscular weakness, inability to walk straight, and so forth. At 10:30 the blood sugar was 0.05 per cent. The symptoms were more pronounced at this point and muscular twitchings had developed. At 10:45 the blood sugar was 0.050 per cent. The symptoms were very pronounced; twitchings involving whole groups of muscles were present, and on tapping the animal with the palm of the hand, symptoms like faint convulsions occurred. At this time 0.5 gram of glucose for each kilogram of body weight, or 15 cc. of a 50 per cent solution, was injected. There was instant recovery. At 10:50 the blood sugar was 0.203 per cent; at 11:00, 0.127 per cent; at 11.15, 0.078 per cent; and at 11:30, 0.050 per cent. The animal had remained in good condition following the administration of glucose, but just before taking this blood specimen definite symptoms of muscular weakness were noted. At 11:45 the blood sugar was 0.044 per cent, and the symptoms were more pronounced, the animal being unable to stand. At 12:00 the blood sugar was 0.039 per cent. The animal showed very marked symptoms, including muscular twitchings. Five-tenths of a gram of glucose for each kilogram of body weight was injected, with instant recovery. At 12:05 the blood sugar was 0.218 per cent; at 12:15, 0.126 per cent; at 12:30, 0.096 per cent, and at 12:45, 0.052 per cent. The animal began to develop definite symptoms. At 1:00 the blood sugar was 0.027 per cent. The animal could not walk, and showed marked muscular twitchings. One cubic centimeter of a 1:1000 adrenalin solution was injected subcutaneously, but no change in the condition of the animal ensued. At 1:15 the blood sugar was 0.043 per cent; at 1:30, 0.043 per cent. The animal was practically moribund at this time, and another injection of 9.5 grams of glucose for each kilogram of body weight was given. At 3:10 the blood sugar was 0.042 per cent. At 3:15 the animal again showed symptoms. Subsequent procedures were not pertinent to the experiment. Necropsy was performed, but nothing was noted which would invalidate the results of this experiment.

Protocol 3. Dog F241. Experiment 251. Male, adult bull terrier weighing 15.3 kgm. March 6, 1922, a reverse Eck fistula was made. April 26, 1922, the dog weighed 14.9 kgm., and the portal vein was ligated. February 26, 1923, the animal was still in excellent condition. The following experiment was performed.

After having fasted for 16 hours, the dog weighed 13.3 kgm. At 8:30 a.m. the first specimen of the blood was taken, and the blood sugar was found to be 0.129 per cent; at 9:00 it was 0.139 per cent; at 9:30, 0.112 per cent, and at 10:00, 0.101 per cent. At this time 1 unit of insulin for each kilogram of body weight, or a total of 1.3 cc. of H/10, no. 729748, was injected intravenously. At 10:15, the fifth specimen of blood was taken; the blood sugar was 0.055 per cent; at 10:30 it was 0.045 per cent, and at 10:45, 0.044 per cent. The animal showed slight symptoms, preferring to lie down, and slight muscular weakness and slight twitchings in the shoulder muscles were noted. At 11:00 the blood sugar was 0.044 per cent. No change in the condition of the animal was noted. At 11:15 the blood sugar was 0.045 per cent. The animal remained in practically the same condition; the symptoms had not progressed. At 11:30 the blood sugar was 0.051 per cent, and at 11:45, 0.071 per cent. The transitory symptoms had practically disappeared. At 12:00 the blood sugar was 0.068 per cent; at 12:15 p.m., 0.071; at 12:30, 0.089 per cent; at 12:45, 0.092 per cent; at 1:00, 0.101 per cent; at 1:15, 0.099 per cent; at 1:30, 0.113 per cent; at 2:00, 0.109 per cent; at 2:30, 0.118 per cent; at 3:30, 0.126 per cent; and at 4:30, 0.109 per cent.

It will be noted that the insulin in this animal produced a very profound depression of blood sugar, but the symptoms were transitory and never very severe. The animal was always able to walk.

The animal was next placed on the regular kennel diet until March 1, when a second experiment was performed as follows: After having fasted for 16 hours, the dog weighed 13.6 kgm. At 8:30 a.m. the twenty-third specimen of blood was taken and the blood sugar was found to be 0.111 per cent. The animal was etherized and the liver removed. The ether was started at 8:30 and the first incision made at 8:37; the circulation was shut off at 8:42 and the liver removed at 8:45. The ether was withdrawn at 9:02. The animal weighed 12.5 kgm. following the operation. The liver weighed 407 grams. The operation was difficult because of a short portal vein and many adhesions. There was more hemorrhage than usually occurs following this operation, although the amount of blood lost was less than 75 cc. At 9:00 the blood sugar was 0.12 per cent; at 9:15, 0.096 per cent; at 9:30, 0.105 per cent; at 9:45, 0.099 per cent, and at 10:00, 0.091 per cent. The animal had recovered from the immediate effects of the operation. The same amount of insulin was injected as in the preceding experiments. At 10:15 the blood sugar had dropped to 0.076 per cent, and at 10:30, to 0.053 per cent. The animal began to show symptoms at this time. At 10:45 the blood sugar was 0.057 per cent. At this time the animal was unable to stand, and muscular twitchings began and became progressively worse, so that at 10:50 it was considered advisable to administer glucose. Accordingly, 0.5 gram of glucose for each kilogram of body weight, or 12.5 cc. of a 50 per cent solution, was administered. Immediately after the administration of the glucose the animal had a convulsion which was very marked. The animal fully recovered, but very slowly. At 10:55 the blood sugar was 0.195 per cent; at 11:05, 0.145 per cent; at 11:20, 0.107 per cent; at 11:50, 0.069 per cent, and at 12:20 p.m., 0.048 per cent. The animal had again developed symptoms and become flaccid. Following an injection of 0.5 gram of glucose for each kilogram of body weight, there was good recovery. At 2:00 the blood sugar was 0.07 per cent. Although typical symptoms were not present, the animal was not in very good condition, and another

injection of glucose was given. Glucose was again given at 3:30, and the animal was killed by bleeding under ether at 4:50. A specimen of blood was taken at this time and the blood sugar was found to be 0.061 per cent. Necropsy was performed immediately, and nothing was found which would invalidate the results of the experiment.

Protocol 4. Dog F488. Experiment 595. Male, adult mongrel, weighing 18.5 kgm. May 30, 1922, a reverse Eck fistula was made. October 9, 1922, the dog weighed 19.2 kgm., and the portal vein was ligated. March 2, 1923, the animal was still in excellent condition, and the following experiment was performed. After having fasted for 16 hours the dog weighed 17.5 kgm. At 8:30 a.m. the first specimen of blood was taken; the blood sugar was found to be 0.10 per cent; at 9:00 it was 0.098 per cent; at 9:30, 0.103 per cent; and at 10:00, 0.115 per cent. At this time 1 unit of insulin for each kilogram of body weight, or a total of 1.75 cc. of no. 729748, was injected intravenously. At 10:15 the blood sugar was 0.075 per cent; at 10:30, 0.054 per cent, and at 10:45, 0.055 per cent. Symptoms were noted at this time. At 11:00 the blood sugar was 0.048 per cent. The animal showed symptoms and seemed dazed, but was able to walk with a staggering gait. At 11:04 the animal went into violent convulsions, and death seemed imminent. Marked rigidity of the legs, retraction of the head, lolling of the tongue with salivation, and difficult respiration were noted. At 11:05 the blood sugar had dropped to 0.041 per cent. At this time glucose was injected, and after 5 cc. had been administered, the symptoms diminished. One minute after the injection was completed the animal could walk and the symptoms had disappeared. At 11:00 the blood sugar had risen to 0.205 per cent; at 11:20, it was 0.143 per cent; at 11:35, 0.086 per cent; at 12:05 p.m., 0.052 per cent; at 12:35, 0.060 per cent; at 1:05, 0.073 per cent; at 1:35, 0.110 per cent; at 2:35, 0.099 per cent, and at 3:35, 0.103 per cent. The experiment was discontinued at this time.

March 6, 1923, a second experiment was undertaken. After having fasted for 16 hours the dog weighed 17.5 kgm. At 8:30 a.m. a specimen of the blood was taken and the blood sugar was found to be 0.115 per cent. The animal was etherized at 8:30, the first incision made at 8:40, the circulation shut off at 8:50 and the liver removed at 8:58. The ether was withdrawn at 9:05. The animal weighed 16.6 kgm. following the operation. The liver weighed 377 grams. The amount of blood lost was insignificant. At 9:00 the blood sugar was 0.126 cent; at 9:30, 0.099 per cent; and at 10:00, 0.097 per cent. The animal had recovered and was in good condition. The same amount of insulin was injected as in the preceding experiment. At 10:15 the blood sugar was 0.073 per cent; at 10:30, 0.052 per cent, and at 10:45, 0.05 per cent. The animal showed marked muscular weakness. At 11:00 the blood sugar was 0.045 per cent, and the animal was unable to stand. At 11:15 the blood sugar was 0.04 per cent. The animal was in a critical condition, and 0.5 gram of glucose for each kilogram of body weight, or a total of 16.6 cc. of a 50 per cent solution, was injected. The animal walked one minute after injection. At 11:20 the blood sugar was 0.227 per cent; at 11:30, 0.158; at 11:45, 0.103 per cent, and at 12:15 p.m., 0.056 per cent. The animal was still in good condition. At 12:45 the blood sugar had dropped to 0.05 per cent. The animal could not stand at this time, but was not in a serious condition. The same amount of glucose was again injected. At 4:30 the blood sugar was 0.053 per cent. The animal showed slight weakness. At 5:30 the animal could still

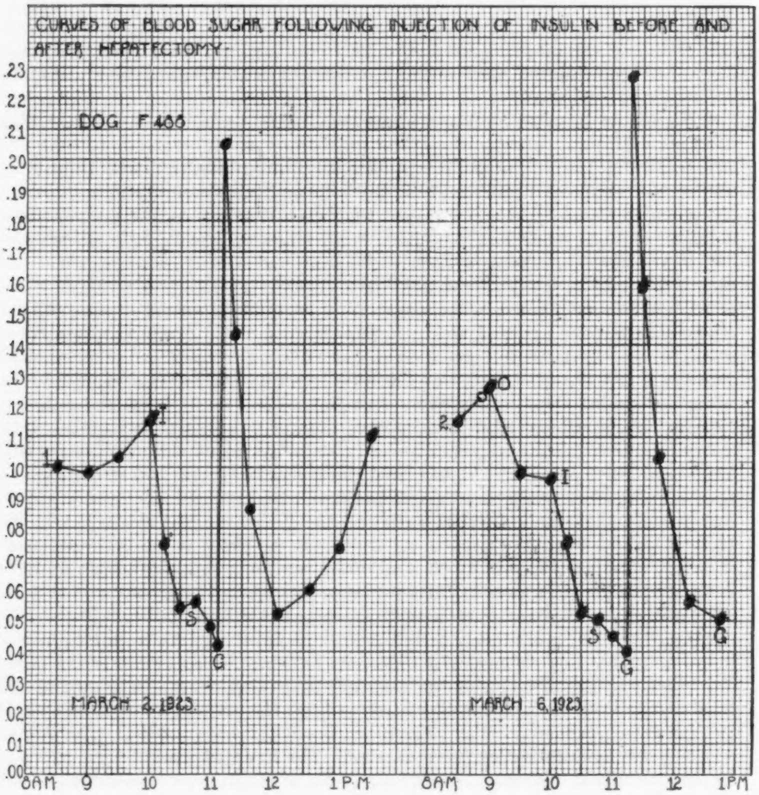

Fig. 2. Curves of blood sugar following the intravenous injection of 1 unit for each kilogram of body weight of insulin before (curve *1*) and after (curve *2*) hepatectomy. In the intact animal the insulin produced a profound decrease in blood sugar, and symptoms developed making it necessary to inject glucose (0.5 gram for each kilogram of body weight). This restored the animal immediately to normal, and although an hour after the administration of glucose the blood sugar was again low, recovery quickly occurred without more glucose. After the liver had been removed, the hypoglycemic action of insulin was the same as in the intact animal; the same symptoms developed at practically the same blood sugar level and the administration of the same amount of glucose produced the same quick response, but the effect was only transitory. Note that the two curves are practically identical, except in regard to the recovery phase. Symbols are same as in figure 1 (protocol 4).

stand. This phase of the experiment was discontinued. Necropsy revealed nothing which would invalidate the results of the experiment.

Protocol 5. Dog F580. Experiment 731. Male, adult, bull terrier weighing 12.1 kgm. A true Eck fistula was made July 5, 1922. March 16, 1923, the dog weighed 9.6 kgm. It was fasted 16 hours, at the end of which (8:30 a.m.) the first specimen of blood was taken, and the blood sugar was found to be 0.128 per cent; at 9:00 it was 0.106 per cent, at 9:30, 0.112 per cent, and at 10:00, 0.104 per cent. At this time 0.25 unit of insulin no. 729753 for each kilogram of body weight, or a total of 0.24 cc., was injected. At 10:15 the fifth specimen of blood

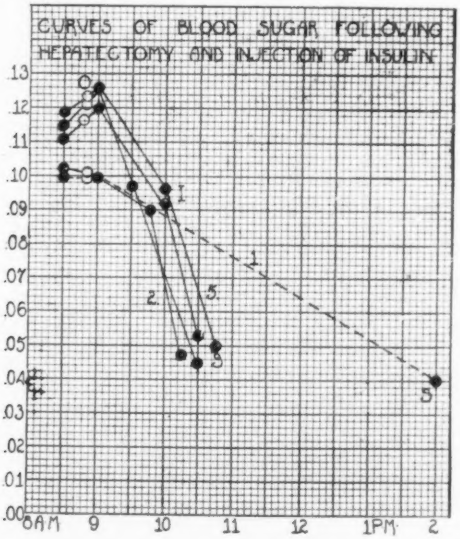

Fig. 3. Curve *1* shows the average change in blood sugar following removal of the liver in the dog. Curves *2* to *5* show the changes following the injection of insulin after hepatectomy. The effect of the insulin is well shown by the more precipitate decrease in blood sugar. *O*, removal of liver; *I*, injection of insulin, and *S*, development of symptoms.

was taken and the blood sugar was found to be 0.057 per cent. At 10:30 it was 0.043; at 10:45, 0.047 per cent; at 11:00, 0.053 per cent; at 11:15, 0.055; at 11:30, 0.053 per cent; at 11:45, 0.073 per cent; at 12:00, 0.084 per cent; at 2:00 p.m., 0.096 per cent; and at 4:00, 0.110 per cent. The animal appeared normal. March 19, 1923, the animal weighed 9.4 kgm. At 8:30 a.m. the fifteenth specimen of blood was taken; the blood sugar was 0.096 per cent. At 8:35 the animal was anesthetized; the first incision was made at 8:45; a portion of the liver, weighing 154 grams, was removed at 8:50; ether was withdrawn at 9:02, when the last stitch was taken. The animal weighed 8.9 kgm. following operation. At 9:00 the six-

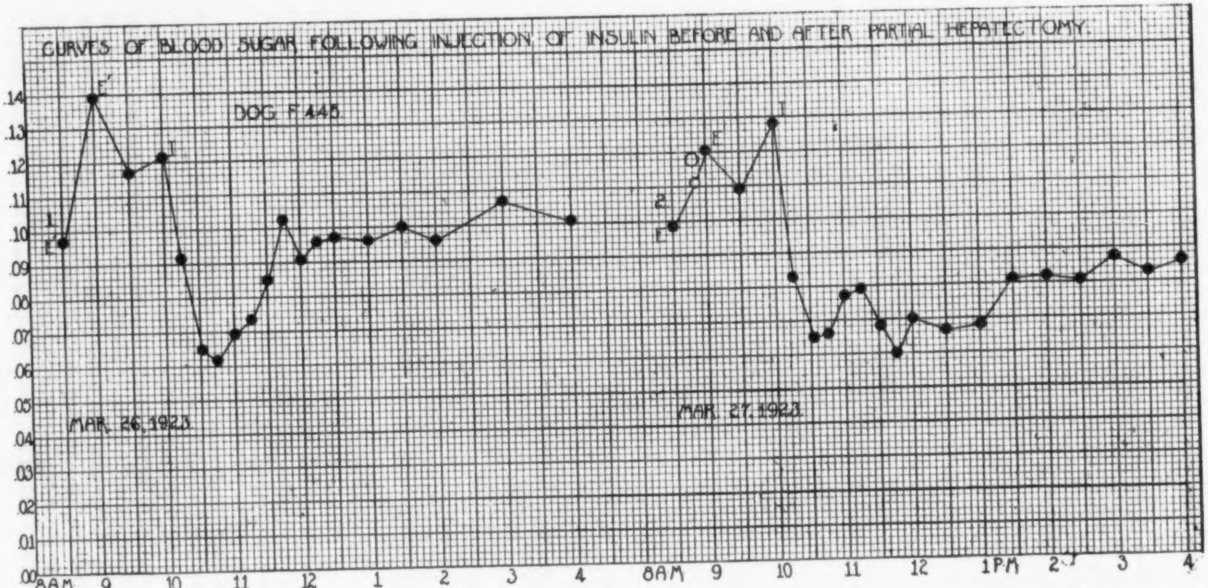

Fig. 4. Curves of blood sugar following the intravenous injection of 0.16 unit for each kilogram of body weight of insulin after anesthesia and before (curve *1*) and after (curve *2*) partial (77 per cent) hepatectomy. In order to make the comparison of the curve more complete, ether was administered (E - E^1) before the injection of insulin in the control experiment. The hypoglycemic action of insulin was the same following each injection; symptoms did not develop in either instance, but the recovery of the blood sugar level was not complete after the loss of liver tissue (protocol 6).

teenth specimen of blood was taken; the blood sugar was 0.153 per cent. At 9:30 it was 0.136 per cent; at 10:00, 0.137 per cent. At this time, the animal had recovered from the anesthesia. One-fourth unit of insulin for each kilogram of body weight, or a total of 0.22 cc. was injected. At 10:15 the blood sugar was 0.121 per cent; at 10:30, 0.101 per cent; at 10:45, 0.081 per cent; at 11:00, 0.081 per cent; at 11:00, 0.081 per cent; at 11:15, 0.07 per cent; at 11:30, 0.066 per cent; at 11:45, 0.059 per cent; at 12:00 m., 0.057 per cent; at 12:15 p.m., 0.053 per cent; at 12:30, 0.05 per cent; at 1:00, 0.06 per cent; at 1:30, 0.064 per cent; at 2:00, 0.088 per cent; at 2:30, 0.079 per cent; at 3:00, 0.091 per cent; at 3:30, 0.09 per cent; and at 4:00, 0.097 per cent. At 8:15 a.m., March 31, three days after operation, the animal died. The portion of the liver remaining at autopsy weighed 110 grams.

Protocol 6. Dog F448. Experiment 554. Male, adult, fox terrier, weighing 13.4 kgm. A true Eck fistula was made May 22, 1922. March 6, 1923, the dog weighed 14.5 kgm. March 13, it weighed 14.8 kgm., and March 26, 14.4 kgm. The animal had been fasted 16 hours. At 8:30 a.m. the first specimen of the blood was taken and the blood sugar was found to be 0.098 per cent. At 8:35 the animal was etherized. At 9:00 the blood sugar was 0.139 per cent. At 9:05 the anesthetic was withdrawn. At 9:30 the blood sugar was 0.117 per cent, and at 10:00, 0.122 per cent. At this time one-sixth unit of insulin no. 729753 for each kilogram of body weight or a total of 0.24 cc. was injected. At 10:15 the blood sugar was 0.091 per cent, at 10:30, 0.065 per cent; at 10:45, 0.062 per cent; at 11:00, 0.07 per cent; at 11:15, 0.074 per cent; at 11:30, 0.085 per cent; at 11:45, 0.103 per cent; at 12:00, 0.088 per cent; at 12:15, 0.096 per cent; at 12:30, 0.097 per cent; at 1:00, 0.096 per cent; at 1:30, 0.100 per cent; at 2:00, 0.096 per cent; at 3:00, 0.107 per cent; and at 4:00, 0.101 per cent. At this time the animal was fed milk, and then fasted 16 hours. March 27, at the end of the fast, the animal weighed 14.2 kgm. At 8:30 a.m. a specimen of the blood was taken, and the blood sugar was 0.109 per cent. The animal was etherized; the first incision was made at 8:40 and a portion of the liver, weighing 255 grams, was removed at 8:45. Ether was withdrawn at 8:50 and the last stitch was made at 9:00. The animal weighed 13.9 kgm. after the operation. At 9:00 the blood sugar was 0.121 per cent; at 9:30, 0.119 per cent; and at 10:00, 0.129 per cent. One-sixth unit of insulin no. 729753 for each kilogram of body weight, or a total of 0.23 cc., was injected. The animal was very restless. At 10:15 the blood sugar was 0.083 per cent, and at 10:30, 0.065 per cent. The animal vomited. At 10:45 the blood sugar was 0.066 per cent; at 11:00, 0.077 per cent; at 11:15, 0.079 per cent; at 11:30, 0.068 per cent; at 11:45, 0.063 per cent; at 12:00, 0.060 per cent; at 12:15, 0.07 per cent; at 12:30, 0.067 per cent; at 1:00, 0.068 per cent; at 1:30, 0.081 per cent; at 2:00, 0,082 per cent; at 2:30, 0.081 per cent; at 3:00, 0.088 per cent; at 3:30, 0.083 per cent; and at 4:00, 0,086 per cent. The animal died about 20 hours after operation. The amount of liver remaining at time of operation weighed 75 grams.

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